PTO/SB/05 (2/98)

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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	04983.0220.00US00/38-10(15494)A
First Named Inventor or Application Identifier	MCININCH
Title	Computational Nucleic Acid Coding and Feature Analysis
Express Mail Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents

ADDRESS TO: Commissioner for Patents
Box Patent Application
Washington, DC 20231

1. [X] *Fee Transmittal Form (Form PTO-1082)
(Submit an original and a duplicate for fee processing)

2. [X] Specification [*Total Pages* 84]
(preferred arrangement set forth below)
- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R&D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claims
- Abstract of the Disclosure

3. [X] Drawing(s) *(35 USC 113)* [*Total Sheets* 11]

4. Oath or Declaration [*Total Pages* 2]
a. [X] Newly executed (original or copy)
b. [ ] Copy from a prior application (37 CFR 1.63(d))
(for continuation/divisional with Box 17 completed)
[Note Box 5 below]
i. [ ] DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

5. [ ] Incorporation By Reference *(useable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. [ ] Microfiche Computer Program *(Appendix)*

7. Nucleotide and/or Amino Acid Sequence Submission
(if applicable, all necessary)
a. [X] Computer Readable Copy
b. [X] Paper Copy (identical to computer copy)
c. [X] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 CFR 3.73(b) Statement *(when there is an assignee)* [X] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [X] Information Disclosure Statement (IDS)/PTO-1449 [X] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) (Two)
(should be specifically itemized)
14. [ ] *Small Entity Statement(s) [ ] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s)
(if foreign priority is claimed)
16. [ ] Other:

*NOTE FOR ITEMS 1 & 14 IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C F R § 1 27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C F R § 1 28)

17. **If a CONTINUING APPLICATION**, *check appropriate box and supply the requisite information:*
[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: /
Prior Application Information: Examiner: Group/Art Unit:

18. CORRESPONDENCE ADDRESS

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Signature		*Date*	October 30, 2000

Burden Hour Statement This form is estimated to take 0 2 hours to complete Time will vary depending upon the needs of the individual case Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231 DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

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Attorney Docket No. 04983.0220.00US00/38-10(15494)A

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of
Inventors: James D. MCININCH
For: **Computational Nucleic Acid Coding and Feature Analysis**

Enclosed are:

xx 11 sheets of informal drawings.
_ An assignment of the invention to _____
A certified copy of a _____application.
_ An associate power of attorney.
_ A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27.
xx Executed Combined Declaration and Power of Attorney for Patent Application.

The filing fee has been calculated as shown below:

	(Col. 1)	(Col. 2)	SMALL ENTITY			OTHER THAN A SMALL ENTITY	
FOR	NO. FILED	NO. EXTRA	RATE	FEE	OR	RATE	FEE
BASIC FEE				$ 355.00	OR		$ 710.00
TOTAL CLAIMS	44 -20 =	24	x 9 =		OR	x 18 =	432.00
INDEP. CLAIMS	11 -3 =	8	x 40 =		OR	x 80 =	640.00
☐ MULTIPLE DEPENDENT CLAIM PRESENTED			+ 135 =		OR	+ 270 =	
*If the difference in Col. 1 is less than zero, enter "0" in Col. 2			TOTAL		OR	TOTAL	$1,782.00

_ Please charge my Deposit Account No. 08-3038 in the amount of $_____. A duplicate copy of this sheet is attached.

_ Please charge my American Express credit card in the amount of $_____. A form PTO-2038, Credit Card Payment Form is attached in duplicate.

xx A check in the amount of $1,782.00 to cover the basic filing fee and excess claims fee is enclosed.

xx The U.S. Patent and Trademark Office is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 08-3038 referencing docket number 04983.0220.00US00/38-10(15494)A. A duplicate copy of this sheet is attached.

xx Any additional filing fees required under 37 CFR 1.16.
xx Any patent application processing fees under 37 CFR 1.17.

_ The U.S. Patent and Trademark Office is hereby authorized to charge payment of the following fees during the pendency of this application or credit any overpayment to Deposit Account No. 08-3038 referencing docket number *. A duplicate copy of this sheet is enclosed.

_ Any patent application processing fees under 37 CFR 1.17
_ The issue fee set in 37 CFR 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 CFR 1.311(b).
_ Any filing fees under 37 CFR 1.16 for presentation of extra claims.

Date October 30, 2000

David R. Marsh (Reg. No. 41,408)
Andrew S. Brenc (Reg. No. 45,534)

HOWREY SIMON ARNOLD & WHITE
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A LIMITED LIABILITY PARTNERSHIP

October 30, 2000

Box Patent Application

Commissioner for Patents
Washington, D.C. 20231

Re: U.S. Non-Provisional Patent Application
Appl. No. To be assigned; Filed: Herewith
For: **Computational Nucleic Acid Coding and Feature Analysis**
Inventor: James D. MCININCH
Our Ref: 04983.0220.00US00/38-10(15494)A

Sir:

The following documents are forwarded herewith for appropriate action by the U.S. Patent and Trademark Office:

1. Utility Patent Application Transmittal Form PTO/SB/05;

2. U.S. Utility Patent Application entitled:

Computational Nucleic Acid Coding and Feature Analysis

and naming as inventors: James D. MCININCH

the application consisting of:

a. A specification containing:

(i) 64 pages of description prior to the claims;

(ii) 12 pages of claims (44 claims);

(iii) a one (1) page abstract;

b. 11 sheets of drawings: (Figures 1-7, 8a-8d, 9a, 9b, and 10a-10c);

c. Sequence listing comprising 7 pages;

3. A computer readable copy of the sequence listing;

4. Statement Regarding Sequence Submission;

5. An original executed Combined Declaration and Power of Attorney;

6. Form PTO-1082 (in duplicate);

7. Information Disclosure Statement;

8. Form PTO-1449 (3 pages) with 7 attached documents;

9. Two (2) return postcards; and

10. Our check No. 339008 for $1,782.00 to cover:

$ 710.00 Filing fee for patent application; and
$ 1,072.00 Fee for excess claims.

It is respectfully requested that, of the two attached postcards, one be stamped with the filing date of these documents and returned to our courier, and the other prepaid postcard be stamped with the filing date and unofficial application number and returned as soon as possible.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the sequence listing and the computer readable copy of the sequence listing submitted herewith in the above application are the same.

The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 08-3038 referencing docket number 04983.0220.00US00/38-10(15494)A. A duplicate copy of this letter is enclosed.

Respectfully submitted,

Andrew S. Brenc

David R. Marsh (Reg. No. 41,408)
Andrew S. Brenc (Reg. No. 45,534)

Enclosures

Computational Nucleic Acid Coding and Feature Analysis

Field of the Invention

The present invention is in the field of bioinformatics, particularly as it pertains to gene prediction. More specifically, the invention relates to the probabilistic analysis of nucleic acid sequences for the determination of coding features, including determination of state probabilities for each nucleotide in a nucleic acid sequence, determination of coding strand, determination of open reading frame extent, determination of insertion and deletion location, determination of exon location, and determination of protein sequence.

Background of the Invention

Advances in techniques for sequencing long stretches of genomic deoxyribonucleic acid (DNA) have allowed investigators to collect vast nucleic acid sequence data rapidly. These advances, combined with initiatives to sequence the entire human genome and the genomes of several other species, have created a need for the rapid identification of genes on long stretches of sequenced DNA. Conventional gene location techniques, such as cDNA hybridization, are effective at locating transcribed genes, but are time-consuming and costly.

An alternative for locating genes on DNA that has not otherwise been analyzed for potential coding regions involves using statistical detection methods. Such methods conventionally include using probability models to predict where in a DNA sequence a gene is located. The theoretical nucleic acid sequence probabilities can be determined through analysis of known coding regions in the organism of interest. Once theoretical nucleic acid sequence probabilities are determined, nucleic acid sequences in unannotated regions of DNA in the same or a similar organism can be statistically compared to the theoretical nucleic acid sequence probabilities. If the similarity is sufficient, the investigator is notified that a coding sequence exists. Conventional cloning techniques can then be used to isolate the putative gene and check for transcription.

One type of statistical detection method searches DNA by content. In such content-based models, highly conserved regions of DNA that are common to all genes are located. If a conserved region of DNA is found, then the nucleic acid sequence associated with the conserved region can be compared with known genes. Such comparisons, which can be done with nucleic

acid sequence comparison programs such as BLAST, are inefficient to run, however, and content-based searches therefore have limited desirability.

A second type of statistical detection method searches DNA by signal. This type of searching involves using probability models to predict whether DNA fragments within a larger nucleic acid sequence are coding. Early searching by signal programs, such as TestCode and Grail, relied on statistical variations within coding regions of DNA, including codon frequency, local nucleic acid sequence composition, codon preference measures, heuristics based on oligonucleotide frequency variations, and measures of nucleic acid sequence complexity.

Beyond simple gene detection, there is also a need for the determination of other coding features, such as the location of intron/exon boundaries in eukaryotic organisms and the location of insertions or deletions. The program GENSCAN (Burge, C. and Karlin, S. (1997) Prediction of Complete Gene Structures in Human Genomic DNA. *J. Mol. Biol.* 268, 78-94), for example, predicts exon location with local state probabilities based on oligonucleotide usage. GENSCAN, however, also depends on non-local nucleic acid sequence characteristics, which make the program very sensitive to sequencing errors and genes containing alternative splicing strategies.

One statistical model that avoids the problems caused by dependence on non-local nucleic acid sequence characteristics is the inhomogeneous Markov model. An inhomogeneous Markov model depends upon local probabilities, and is not therefore sensitive to sequencing errors or genes with alternative splicing strategies. The inhomogeneous Markov model is "inhomogeneous" because it determines the state probabilities for a given nucleotide in multiple reading frames rather than in a single reading frame. GeneMark, for example, is a computer program that uses the inhomogeneous Markov model to locate genes.

The GeneMark gene prediction algorithm was developed in several steps. A series of three publications demonstrated that inhomogeneous Markov models were useful tools for gene prediction (*see* Borodovsky, M., Sprizhitsky Yu., Golovanov E. and Alexandrov A. (1986) *Statistical Patterns in Primary Structures of Functional Regions in the E. Coli Genome: I. Oligonucleotide Frequencies Analysis*, Molecular Biology, 20, 826-833, Borodovsky, M., Sprizhitsky Yu, Golovanov E. and Alexandrov A. (1986) *Statistical Patterns in Primary Structures of Functional Regions in the E. Coli Genome: II. Non-homogeneous Markov Models*, Molecular Biology, 20, 833-840, Borodovsky, M., Sprizhitsky Yu., Golovanov E. and

Alexandrov A. (1986) *Statistical Patterns in Primary Structures of Functional Regions in the E. Coli Genome: III. Computer Recognition of Coding Regions*, Molecular Biology, 20, 1145-1150, all of which are herein incorporated by reference in their entirety). The GeneMark method was based on an inhomogeneous Markov model and was described in 1993 (*see* Borodovsky, M. and McIninch J. (1993) GeneMark, *Parallel Gene Recognition for both DNA Strands*, Computers & Chemistry, 17, 123-133, and Borodovsky, M. and McIninch J. (1993) BioSystems v30, pp. 161-171, both of which are herein incorporated by reference in their entirety). The capabilities of the GeneMark program were subsequently investigated (*see* James D. McIninch, *Prediction of Protein Coding Regions in Unannotated DNA sequences Using an Inhomogeneous Markov Model of Genetic Information Encoding* (1997) (Ph.D. dissertation, Georgia Institute of Technology, on file with the Georgia Institute of Technology Library, which is herein incorporated by reference in its entirety).

Conventional programs using inhomogeneous Markov models, however, are limited to a defined probabilistic model for determining probability, and cannot be tailored by the investigator to better suit the nucleic acid sequence under study if information about that nucleic acid sequence is already available. Further, conventional implementations do not allow for the efficient and accurate detection of other nucleic acid sequence features.

What is needed in the art is a method of determining state probabilities for a nucleic acid sequence having some known characteristics, where the method is insensitive to frameshift insertions or deletions, and compatible methods for detecting other nucleic acid sequence features in known or unknown nucleic acid sequences.

Summary Of The Invention

The present invention relates to the probabilistic analysis of nucleic acid sequences for the determination of coding features, including determination of state probabilities for each nucleotide in a nucleic acid sequence, determination of coding strand, determination of open reading frame extent, determination of insertion and deletion location, determination of exon location, and determination of protein sequence. Described herein are methods, devices, and systems for analyzing the information content in nucleic acids.

The present invention includes and provides a method for determining a probability for one or more states for a nucleotide in a nucleic acid sequence, comprising: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in the nucleic acid sequence; b) determining transition probabilities for each of the states for nucleotides within the nucleic acid sequence following the initial oligonucleotide; c) determining a probability for the nucleic acid sequence for each of the states; and, d) determining a probability for each of the states for the nucleotide based upon the probability of the nucleic acid sequence and a bias.

The present invention includes and provides a method for determining a probability for one or more states for a nucleotide in a nucleic acid sequence, comprising: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in the nucleic acid sequence; b) determining transition probabilities for each of the states for nucleotides within the nucleic acid sequence following the initial oligonucleotide; c) determining a probability for the nucleic acid sequence for each of the states; and, d) determining a probability for each of the states for the nucleotide based upon the probability of the nucleic acid sequence, wherein the determining a probability for each of the states is capable of accepting a bias.

The present invention includes and provides a method for determining a probability for each of one or more states for more than one nucleotide in a nucleic acid sequence comprising: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in a window of a first nucleotide; b) determining transition probabilities for each of the states for nucleotides within the window following the initial oligonucleotide; c) determining a probability for the window for each of the states; d) determining a probability for each of the states for the nucleotide based upon the probability for the window and a bias; and, e) repeating steps a) through d) for each remaining nucleotide in the nucleic acid sequence.

The present invention includes and provides a method for determining strand coding of a nucleic acid sequence based upon a bias, comprising: a) determining a probability of each of one or more states for each nucleotide in the nucleic acid sequence, wherein each of the states is either a positive strand state or a negative strand state; b) summing the probabilities of the positive strand states for each of the nucleotides to produce a sum of probabilities for positive

states; c) summing the probabilities of the negative strand states for each of the nucleotides to produce a sum of probabilities for negative states; and, d) deciding one of i) coding is mixed or not detectable if a first function of the sum of probabilities for positive states and the sum of probabilities for negative states is less than a threshold value; ii) coding is on the positive strand if a second function of the sum of probabilities for positive states is greater than a third function of the sum of probabilities for negative states and the first function is not less than the threshold value; and iii) coding is on the negative strand if the second function of the sum of probabilities for positive states is not greater than the third function of the sum of probabilities for negative states and the first function is not less than the threshold value.

The present invention includes and provides a method for determining the extent of an open reading frame within a nucleic acid sequence based upon a bias, comprising: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence, wherein each of the states is either a coding state or a noncoding state; b) determining the coding strand of the nucleic acid sequence; and, c) determining the points within the nucleic acid sequence in the coding strand at which the sum of the probabilities of the coding states for each nucleotide drops below a first threshold value for a number of nucleotides greater than a second threshold value, wherein ends of the open reading frame are indicated at the points.

The present invention includes and provides a method for determining the location of insertions and deletions within a nucleic acid sequence, comprising: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) setting a length for a window; c) determining which state has a maximum mean probability for the nucleic acid sequence on a first side of a middle nucleotide in the window, wherein the window begins at a first nucleotide; d) determining which state has a maximum mean probability for the nucleic acid sequence on a second side of the middle nucleotide in the window; e) determining that a deletion or insertion occurred at the middle nucleotide if i) the state with the maximum mean probability on the first side of the middle nucleotide is different from the state with the maximum mean probability on the second side of middle nucleotide, and ii) either an average of hypothetical state probabilities for the window with an insertion at the middle nucleotide or an average of hypothetical state probabilities for the window with a deletion at the middle

nucleotide is greater than a sum of the middle nucleotide's coding states probabilities; and, f) repeating steps c) through e) for each remaining nucleotide in the nucleic acid sequence after the first nucleotide, wherein the window begins at each remaining nucleotide in turn.

The present invention includes and provides a method for determining exon location within a nucleic acid sequence, comprising a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or noncoding state; b) determining the coding strand of the nucleic acid sequence; c) determining the extent of an open reading frame within the nucleic acid sequence; d) classifying each nucleotide in a coding class or a noncoding class based on a most probable state for the coding strand; e) reclassifying each nucleotide according to defined rules; and, f) determining that regions of the nucleic acid sequence in the coding class are exons.

The present invention includes and provides a program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine a probability for each of one or more states for a nucleotide in a nucleic acid sequence, the method steps comprising: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in the nucleic acid sequence; b) determining transition probabilities for each of the states for nucleotides within the nucleic acid sequence following the initial oligonucleotide; c) determining a probability for the nucleic acid sequence for each of the states; and, d) determining a probability for each of the states for the nucleotide based upon the probability of the nucleic acid sequence and a bias.

The present invention includes and provides a program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine a probability for one or more states for more than one nucleotide in a nucleic acid sequence, the method steps comprising: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in a window of a first nucleotide; b) determining transition probabilities for each of the states for nucleotides within the window following the initial oligonucleotide; c) determining a probability for the window for each of the states; d) determining a probability for each of the states for the nucleotide based upon the probability for the window and a bias; and, e) repeating steps a) through d) for each remaining nucleotide in the nucleic acid sequence.

The present invention includes and provides a program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine strand coding of a nucleic acid sequence, the method steps comprising: a) determining a probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a positive strand state or a negative strand state; b) summing the probabilities of the positive strand states for each of the nucleotides to produce a sum of probabilities for positive states; c) summing the probabilities of the negative strand states for each of the nucleotides to produce a sum of probabilities for negative states; and, d) deciding one of i) coding is mixed or not detectable if a first function of the sum of probabilities for positive states and the sum of probabilities for negative states is less than a threshold value; ii) coding is on the positive strand if a second function of the sum of probabilities for positive states is greater than a third function of the sum of probabilities for negative states and the first function is not less than the threshold value; and iii) coding is on the negative strand if the second function of the sum of probabilities for positive states is not greater than the third function of the sum of probabilities for negative states and the first function is not less than the threshold value.

The present invention includes and provides a program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine the extent of an open reading frame within a nucleic acid sequence, the method steps comprising: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) determining the coding strand of the nucleic acid sequence; and, c) determining the points within the nucleic acid sequence in the coding strand at which the sum of the probabilities of the coding states for each nucleotide drops below a first threshold value for a number of nucleotides greater than a second threshold value, wherein ends of the open reading frame are indicated at the points.

The present invention includes and provides a program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine the location of insertions and deletions within a nucleic acid sequence, the method steps comprising: a) determining the probability of each of one or more states for

each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) setting a length for a window; c) determining which state has a maximum mean probability for the nucleic acid sequence on a first side of a middle nucleotide in the window, wherein the window begins at a first nucleotide; d) determining which state has a maximum mean probability for the nucleic acid sequence on a second side of the middle nucleotide in the window; e) determining that a deletion or insertion occurred at the middle nucleotide if i) the state with the maximum mean probability on the first side of the middle nucleotide is different from the state with the maximum mean probability on the second side of middle nucleotide, and ii) either an average of hypothetical state probabilities for the window with an insertion at the middle nucleotide or an average of hypothetical state probabilities for the window with a deletion at the middle nucleotide is greater than a sum of the middle nucleotide's coding states probabilities; and, f) repeating steps c) through e) for each remaining nucleotide in the nucleic acid sequence after the first nucleotide, wherein the window begins at each remaining nucleotide in turn.

The present invention includes and provides a program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine exon location within a nucleic acid sequence, the method steps comprising: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or noncoding state; b) determining the coding strand of the nucleic acid sequence; c) determining the extent of an open reading frame within the nucleic acid sequence; d) classifying each nucleotide in a coding class or a noncoding class based on a most probable state for the coding strand; e) reclassifying each nucleotide according to defined rules; and, f) determining that regions of the nucleic acid sequence in the coding class are exons.

The present invention includes and provides a computer system for determining a probability for each of one or more states for a nucleotide in a nucleic acid sequence, comprising: an input device for inputting the nucleic acid sequence; a memory for storing the nucleic acid sequence; a processing unit configured for retrieving the nucleic acid sequence and for: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in the nucleic acid sequence; b) determining transition probabilities for each of

the states for nucleotides within the nucleic acid sequence following the initial oligonucleotide; c) determining a probability for the nucleic acid sequence for each of the states; and, d) determining a probability for each of the states for the nucleotide based upon the probability of the nucleic acid sequence and a bias.

The present invention includes and provides a computer system for determining a probability for each of one or more states for more than one nucleotide in a nucleic acid sequence, comprising: an input device for inputting the nucleic acid sequence; a memory for storing the nucleic acid sequence; a processing unit configured for retrieving the nucleic acid sequence and for: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in a window of a first nucleotide; b) determining transition probabilities for each of the states for nucleotides within the window following the initial oligonucleotide; c) determining a probability for the window for each of the states; d) determining a probability for each of the states for the nucleotide based upon the probability for the window and a bias; and, e) repeating steps a) through d) for each remaining nucleotide in the nucleic acid sequence.

The present invention includes and provides a computer system for determining strand coding of a nucleic acid sequence, comprising: an input device for inputting the nucleic acid sequence; a memory for storing the nucleic acid sequence; a processing unit configured for retrieving the nucleic acid sequence and for: a) determining a probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a positive strand state or a negative strand state; b) summing the probabilities of the positive strand states for each of the nucleotides to produce a sum of probabilities for positive states; c) summing the probabilities of the negative strand states for each of the nucleotides to produce a sum of probabilities for negative states; and, d) deciding one of i) coding is mixed or not detectable if a first function of the sum of probabilities for positive states and the sum of probabilities for negative states is less than a threshold value; ii) coding is on the positive strand if a second function of the sum of probabilities for positive states is greater than a third function of the sum of probabilities for negative states and the first function is not less than the threshold value; and iii) coding is on the negative strand if the second function of the sum of probabilities for positive states is not greater than the third function of the sum of probabilities for negative states and the first function is not less than the threshold value.

The present invention includes and provides a computer system for determining the extent of an open reading frame within a nucleic acid sequence, comprising: an input device for inputting a nucleic acid sequence; a memory for storing the nucleic acid sequence; a processing unit configured for retrieving the nucleic acid sequence and for: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) determining the coding strand of the nucleic acid sequence; and, c) determining the points within the nucleic acid sequence in the coding strand at which the sum of the probabilities of the coding states for each nucleotide drops below a first threshold value for a number of nucleotides greater than a second threshold value, wherein ends of the open reading frame are indicated at the points.

The present invention includes and provides a computer system for determining the location of insertions and deletions within a nucleic acid sequence, comprising: an input device for inputting a nucleic acid sequence; a memory for storing the nucleic acid sequence; a processing unit configured for retrieving the nucleic acid sequence and for: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) setting a length for a window; c) determining which state has a maximum mean probability for the nucleic acid sequence on a first side of a middle nucleotide in the window, wherein the window begins at a first nucleotide; d) determining which state has a maximum mean probability for the nucleic acid sequence on a second side of the middle nucleotide in the window; e) determining that a deletion or insertion occurred at the middle nucleotide if i) the state with the maximum mean probability on the first side of the middle nucleotide is different from the state with the maximum mean probability on the second side of middle nucleotide, and ii) either an average of hypothetical state probabilities for the window with an insertion at the middle nucleotide or an average of hypothetical state probabilities for the window with a deletion at the middle nucleotide is greater than a sum of the middle nucleotide's coding states probabilities; and, f) repeating steps c) through e) for each remaining nucleotide in the nucleic acid sequence after the first nucleotide, wherein the window begins at each remaining nucleotide in turn.

The present invention includes and provides a computer system for determining exon location within a nucleic acid sequence, comprising: an input device for inputting a nucleic acid

sequence; a memory for storing the nucleic acid sequence; a processing unit configured for retrieving the nucleic acid sequence and for: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or noncoding state; b) determining the coding strand of the nucleic acid sequence; c) determining the extent of an open reading frame within the nucleic acid sequence; d) classifying each nucleotide in a coding class or a noncoding class based on a most probable state for the coding strand; e) reclassifying each nucleotide according to defined rules; and, f) determining that regions of the nucleic acid sequence in the coding class are exons.

The present invention includes and provides a computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to determine a probability for each of one or more states for a nucleotide in a nucleic acid sequence, the computer program logic comprising means for enabling the processor to perform each of the following steps: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in the nucleic acid sequence; b) determining transition probabilities for each of the states for nucleotides within the nucleic acid sequence following the initial oligonucleotide; c) determining a probability for the nucleic acid sequence for each of the states; and, d) determining a probability for each of the states for the nucleotide based upon the probability of the nucleic acid sequence and a bias.

The present invention includes and provides a computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to determine a probability for each of one or more states for more than one nucleotide in a nucleic acid sequence, the computer program logic comprising means for enabling the processor to perform each of the following steps: a) determining an initial oligonucleotide probability for each of the states for an initial oligonucleotide in a window of a first nucleotide; b) determining transition probabilities for each of the states for nucleotides within the window following the initial oligonucleotide; c) determining a probability for the window for each of the states; d) determining a probability for each of the states for the nucleotide based upon the probability for the window and a bias; and, e) repeating steps a) through d) for each remaining nucleotide in the nucleic acid sequence.

The present invention includes and provides a computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to determine strand coding of a nucleic acid sequence, the computer program logic comprising means for enabling the processor to perform each of the following steps: a) determining a probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a positive strand state or a negative strand state; b) summing the probabilities of the positive strand states for each of the nucleotides to produce a sum of probabilities for positive states; c) summing the probabilities of the negative strand states for each of the nucleotides to produce a sum of probabilities for negative states; and, d) deciding one of i) coding is mixed or not detectable if a first function of the sum of probabilities for positive states and the sum of probabilities for negative states is less than a threshold value; ii) coding is on the positive strand if a second function of the sum of probabilities for positive states is greater than a third function of the sum of probabilities for negative states and the first function is not less than the threshold value; and iii) coding is on the negative strand if the second function of the sum of probabilities for positive states is not greater than the third function of the sum of probabilities for negative states and the first function is not less than the threshold value.

The present invention includes and provides a computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to determine the extent of an open reading frame within a nucleic acid sequence, the computer program logic comprising means for enabling the processor to perform each of the following steps: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) determining the coding strand of the nucleic acid sequence; and, c) determining the points within the nucleic acid sequence in the coding strand at which the sum of the probabilities of the coding states for each nucleotide drops below a first threshold value for a number of nucleotides greater than a second threshold value, wherein ends of the open reading frame are indicated at the points.

The present invention includes and provides a computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a

processor in a computer system to determine the location of insertions and deletions within a nucleic acid sequence, the computer program logic comprising means for enabling the processor to perform each of the following steps: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or a noncoding state; b) setting a length for a window; c) determining which state has a maximum mean probability for the nucleic acid sequence on a first side of a middle nucleotide in the window, wherein the window begins at a first nucleotide; d) determining which state has a maximum mean probability for the nucleic acid sequence on a second side of the middle nucleotide in the window; e) determining that a deletion or insertion occurred at the middle nucleotide if i) the state with the maximum mean probability on the first side of the middle nucleotide is different from the state with the maximum mean probability on the second side of middle nucleotide, and ii) either an average of hypothetical state probabilities for the window with an insertion at the middle nucleotide or an average of hypothetical state probabilities for the window with a deletion at the middle nucleotide is greater than a sum of the middle nucleotide's coding states probabilities; and, f) repeating steps c) through e) for each remaining nucleotide in the nucleic acid sequence after the first nucleotide, wherein the window begins at each remaining nucleotide in turn.

The present invention includes and provides a computer program product comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to determine exon location within a nucleic acid sequence, the computer program logic comprising means for enabling the processor to perform each of the following steps: a) determining the probability of each of one or more states for each nucleotide in the nucleic acid sequence based upon a bias, wherein each of the states is either a coding state or noncoding state; b) determining the coding strand of the nucleic acid sequence; c) determining the extent of an open reading frame within the nucleic acid sequence; d) classifying each nucleotide in a coding class or a noncoding class based on a most probable state for the coding strand; e) reclassifying each nucleotide according to defined rules; and, f) determining that regions of the nucleic acid sequence in the coding class are exons.

The present invention includes and provides a method for determining a probability for one or more states for a nucleotide in a nucleic acid sequence, comprising determining a

probability for each of the states for the nucleotide based upon a probability of the nucleic acid sequence and a bias.

The present invention includes and provides a method for determining a probability for each of one or more states for more than one nucleotide in a nucleic acid sequence comprising: a) determining a probability for each of the states for a first nucleotide in the nucleic acid sequence based upon a probability of a window in which the first nucleotide is located and a bias; and, b) repeating step a) for the remaining nucleotides in the nucleic acid sequence.

Description Of The Figures

Figure 1 is a flow chart representing one embodiment of a method for determining the probability of each of the possible states for a single nucleotide in a nucleic acid sequence;

Figure 2 is a flow chart representing one embodiment of a method for determining the probability of each of the possible states for a multiple nucleotides in a nucleic acid sequence;

Figure 3 is a flow chart representing one embodiment of a method for determining the coding strand of a nucleic acid sequence;

Figure 4 is a flow chart representing one embodiment of a method for determining the extent of an open reading frame within a nucleic acid sequence;

Figure 5 is a flow chart representing one embodiment of a method for determining the location of insertions and deletions within a nucleic acid sequence;

Figure 6 is a flow chart representing one embodiment of a method for determining the extent of exons within a nucleic acid sequence and the protein translation of those exons;

Figure 7 is a flow chart representing one embodiment of a method for determining the extent of exons within a nucleic acid sequence and the protein translation of those exons;

Figure 8a is a schematic representation of a window located at the end of a nucleic acid sequence;

Figure 8b is a schematic representation of a window located at the end of a nucleic acid sequence showing nucleotides near the end of the nucleic acid sequence;

Figure 8c is a schematic representation showing the ends of a nucleic acid sequence being copied to form a hypothetical extension on each end of the nucleic acid sequence;

Figure 8d is a schematic representation of a nucleic acid sequence showing the appended hypothetical extensions;

Figure 9a is a schematic representation of one embodiment of a computer system that can implement the methods of the present invention;

Figure 9b is a schematic representation of one embodiment of a computer system that can implement the methods of the present invention;

Figure 10a is a schematic representation of a genomic sequence of DNA with an aligned expressed sequence tag aligned thereto;

Figure 10b is a schematic representation of a window in a region of DNA when the entire region is in a known coding region; and,

Figure 10c is a schematic representation of a window in a region of DNA when part of the region is known to be coding, and part of the region is known to be noncoding.

Detailed Description Of The Invention

Described herein are methods for determining the state probabilities of one or more nucleotides in a nucleic acid sequence, the coding strand of a nucleic acid sequence, the extent of an open reading frame in a nucleic acid sequence, the location of deletions and insertions in a nucleic acid sequence, the location of exons in a nucleic acid sequence, and the translation of those exons. Also described are program storage devices readable by a machine, tangibly embodying a program of instructions executable by a machine to perform the above methods. Also described are computer systems for implementing the above methods, comprising an input device for inputting a nucleic acid sequence, a memory for storing the nucleic acid sequence, and a processing unit. Also described are computer program products comprising a computer usable medium having computer program logic recorded thereon for enabling a processor in a computer system to perform the above methods.

Definitions:

Nucleic Acid Sequence - As used herein, "nucleic acid sequence" includes a nucleic acid sequence of any nucleic acid as is generally understood in the art. The nucleic acid can be DNA, cDNA, genomic DNA, raw DNA, expressed nucleic acid sequence tags (ESTs), RNA, mRNA,

unprocessed RNA, processed RNA, or any other form of nucleic acid, regardless of whether or not the nucleic acid actually codes for a protein.

Nucleic acid sequences can be derived from any natural or artificial source, including prokaryotic and eukaryotic organisms, and can be at any stage of processing.

It is understood by those skilled in the art that any representation of a nucleic acid sequence is contemplated herein and within the scope of the present invention. That is, while conventionally nucleic acid sequences are represented by the nucleotide or base letters A, T, G, C, U, any alphanumeric or other representation of nucleotide or base nucleic acid sequence, whether digitally represented or otherwise, is within the scope of this invention. Further, nucleic acid sequence notation indicating uncertainty with respect to the identification of one or more bases in a nucleic acid sequence, for example IUB nomenclature such as R=G and A, Y=T and C, etc., can be incorporated into the method described herein and is within the scope of this invention.

Nucleic acid sequences having modified or non-standard bases can be incorporated into the method described herein and are within the scope of this invention. For the purposes of this invention, a nucleic acid sequence of "bases" is an equivalent nucleic acid sequence to the nucleic acid sequence in which the bases are found.

Reading frame – A "reading frame" is one of the possible phases in which one can read a sequence of codons (groups of three nucleotides) that can make up a coding region of DNA or RNA. In a codon the positions in 5' to 3' order are called the "first", "second", and "third" reading frames.

States - The "states" attributable to a nucleotide are the potential permutations of all of the possible reading frames and the two nucleic acid strands included in the probability model being used. A "+" is used to indicate the positive strand, and "-" to indicate the reverse compliment DNA strand. In a preferred embodiment, the possible states of any one nucleotide are positive strand first reading frame (1+), positive strand second reading frame (2+), positive strand third reading frame (3+), negative strand first reading frame (1-), negative strand second reading frame (2-), negative strand third reading frame (3-), positive strand noncoding (N+), and negative

strand noncoding (N-). In another embodiment, the states can be, for example, just the four positive states listed above. Stated symbolically, "f" is an element in the set of states, i.e. $f \in \{1+, 2+, 3+, N+, 1-, 2-, 3-, N-\}$.

Coding State - A "coding state" is any of the states 1+, 2+, 3+, 1-, 2-, or 3-, which indicate coding, i.e. nucleic acids translated into protein.

Noncoding state - A "noncoding state" is either of the states N- or N+, both of which indicate noncoding, i.e. no protein translation.

Sequentially - "Sequentially" means performing a step or series of steps on nucleotides in order as the nucleotides occur in the nucleic acid sequence, in either direction.

State probabilities - The "state probabilities" of a nucleotide within a nucleic acid sequence are a vector of probabilities associated with the given nucleotide being in each of the states.

Window - A "window" is a contiguous and defined number of nucleotides within a nucleic acid sequence. For example, in a nucleic acid sequence having a length of several thousand nucleotides, a window of, again for example, 100 nucleotides can be defined for specific analysis at any place within the larger nucleic acid sequence.

Middle Nucleotide - The "middle nucleotide" in any given nucleic acid sequence or window is the nucleotide found at the numerical middle of the nucleic acid sequence or window, respectively, wherein the length of a nucleic acid sequence or window is the total number of nucleotides in the nucleic acid sequence or window. If the nucleic acid sequence or window has an even number of nucleotides, then the middle nucleotide can be either of the two nucleotides ajacent the numerical middle of the nucleic acid sequence or window. For example, the middle nucleotide in a 101 nucleotide long window is nucleotide number 51, and the middle nucleotide in a 100 nucleotide long window can be either nucleotide number 50 or nucleotide number 51.

Oligonucleotide - An "oligonucleotide" is a a series of contiguous nucleotides with a defined length.

Initial Oligonucleotide - The "initial oligonucleotide" is the oligonucleotide that occurs at the beginning of the nucleic acid sequence or window being examined. Therefore, the first nucleotide in the initial oligonucleotide is also the first nucleotide in the sequence or window.

Transition Probability - A "transition probability" for a given nucleotide is the probability of the nucleotide occurring given the oligonucleotide immediately preceding that nucleotide.

Bias Function - The "Bias Function" is a function that is used to differentialy alter the probability of one or more states of one or more nucleotides in a nucleic acid sequence. For example, if a region of the nucleic acid sequence under study is thought to be a coding region, then the bias function can be used to increase the calculated probability of the coding states for that nucleic acid sequence.

Bias - "Bias" is a set of one or more values that are used in the Bias Function, and is used to alter the probability of one or more states of one or more nucleotides in a nucleic acid sequence.

Filter - A "filter" as used herein is any method or algorithm for unifying and making more homogeneous regions of a nucleic acid sequence that have been classified in disparate states. A filter is used for the purpose of more clearly defining coding region boundaries in a nucleic acid sequence. In a method, a step in which a filter is applied is a "filtering step."

Class - A "class" of nucleotides is a group of nucleotides that are designated as having one state for the purposes of filtering.

Positive Strand and Negative Strand - The terms "positive strand (+)" and "negative strand (-)" represent complementary nucleic acid sequences. The sequence in one strand is defined by the sequence in the complementary strand.

Positive Strand State - A "positive strand state" is any of states 1+, 2+, 3+, N+.

Negative Strand State - A "negative strand state" is any of states 1-, 2-, 3-, N-.

Description

The methods described herein can be performed in any manner that allows for the analysis of the nucleic acid sequence under study and computation of the probabilities associated with that nucleic acid sequence. In a preferred embodiment, the physical nucleic acid sequence, for example a DNA sequence having a contiguous nucleic acid sequence of G, C, T, and A nucleotides, is converted into digital form by, for example, inputting the nucleic acid sequence into a computer system. The computer then processes the nucleic acid sequence using the methods described herein. Any nucleic acid sequence referred to herein can be arranged to have a beginning and an end, and numbered so that the first nucleotide in the nucleic acid sequence is number 1, the next nucleotide in the nucleic acid sequence is number 2, and so on until the end of the nucleic acid sequence. Any other numbering scheme that is useful can be used.

The methods shown in Figures 1-7 are independent, and, although several of the methods described can be utilized together, they can each be performed as independent methods. Further, where one method calls for a step in which one of the other methods can be used for that step, the use of the other method in the step represents only one embodiment, and other methods for performing the step can be used as well.

Any probability model applicable to nucleic acid sequence state probabilities can be used for the probability steps if the output of the probability model sufficiently supports the method, including inhomogeneous Markov models that have fewer than eight states, for example, those having only six or four states. In a preferred embodiment, the inhomogeneous Markov model has eight states. (For a general discussion of various models, see Durbin, et al., Biological Sequence Analysis (1998), which is herein incorporated by reference in its entirety).

Any nucleic acid sequence source can be used, regardless of the accuracy of the nucleic acid sequence relative to the physical molecule it represents, including raw nucleic acid sequence data and nucleic acid sequence data that has been changed or adjusted for other purposes, such as

nucleic acid sequences that have been filtered to improve accuracy, nucleic acid sequences that have been altered to account for known mutations, and nucleic acid sequences that have been engineered in any manner whatsoever, among others. Nucleic acid sequence information produced by automated nucleic acid sequencers can be used, as well as nucleic acid sequence information derived by any conventional sequencing technique, such as dideoxy sequencing, among others. Nucleic acid sequences produced by or from other bioinformatic processing methods or nucleic acid databases can be used, for example, including nucleic acid sequences stored in public access databases such as GenBank. Although nucleic acid sequences with any amount of error can be used, in a preferred embodiment the amount of sequencing error present is less than about 15%, and more preferably is less than about 10%. However, an advantage of the methods of the present invention is that they can utilize lower quality nucleic acid sequences. In this embodiment, the methods of the present invention can utilize nucleic acid sequences where the average sequence accuracy is less than 99%, more preferably less than 95%, more preferably less than 90, 80, or 70%.

The present invention includes the incorporation of bias into probability models that determine state probabilities for one or more nucleotides. The bias is used to alter the statistical probability of one or more states for a nucleotide. A bias of zero, for example, will reduce the probability of a state to zero, while a bias of one will not alter the statistical probability. Values greater than one will increase the statistical probability of a state, while values between zero and one will reduce the statistical probability of a state. Bias can be defined by the investigator in order to influence the probability of states. In a preferred embodiment, bias is defined to alter the probability of states in a manner consistent with existing knowledge of the nucleic acid sequence under study. For example, if a nucleic acid sequence has a region that is strongly suspected to be coding, then the nucleotides in that region can be assigned a large bias for the coding states, and a small bias for the noncoding states. Bias can be incorporated into any conventional statistical model that provides a method for determining state probabilities in order to allow for the biasing of statistical probabilities in that model. In one embodiment, bias can be defined for each state as a number equal to or greater than zero, excluding 1. In this embodiment, the statistical probability of a state will be reduced if the bias is set to a number equal to or greater than zero and less than one, and increased if the bias is set to a number greater than one, and all states are

biases in one direction or the other. In another embodiment, bias can be defined as one for one or more states, and a number other than one for one or more states. In this embodiment, one or more states has a defined bias of one, which results in no biasing of the probability of that state, while one or more states have a defined value equal to or greater than zero, excluding one. In this embodiment, one or more states are biased, and one or more states are not. In a preferred embodiment, the bias is between 0.0 and 0.9 or greater than 1.1.

Figure 1 represents one embodiment of the method of the present invention for determining the state probabilities of a single nucleotide within a nucleic acid sequence. The nucleotide for which the state probabilities are determined can be any nucleotide in the nucleic acid sequence, preferably is a nucleotide close to the middle of the sequence, and in a preferred embodiment the nucleotide is the middle nucleotide in the nucleic acid sequence. It is preferable to determine state probabilities for a nucleotide at or near the middle of the nucleic acid sequence. State probabilities for the nucleotide are determined by first finding the probability of the initial oligonucleotide in the nucleic acid sequence, and then finding the transition probabilities for the remainder of the nucleotides in the nucleic acid sequence. The initial oligonucleotide probability and transition probability information is used to determine the probabilities of each of the states for the entire nucleic acid sequence, and the resulting state probabilities are assigned to the nucleotide. Eight states are described below for Figure 1, but those of skill in the art will readily see that fewer than eight states can be employed.

Referring now to Figure 1, in step 12, the probability that the initial oligonucleotide occurs in each of the states is determined according to equation I:

$$P_f(a_1 \ldots a_k) = \frac{|a_1 \ldots a_k|_f}{N_f} \qquad \text{(I)}$$

where "$a_1 \ldots a_k$" is an initial oligonucleotide of length k, a_1 is the first nucleotide in the oligonucleotide, N_f is the set of all oligonucleotides occurring in the model sample set, and f is an element of the set of states, which, in a preferred embodiment, is {1+,2+,3+,N+,1-,2-,3-,N-}.

The oligonucleotide length is predefined, and can be any length for which probabilities can be reliably generated. Oligonucleotides can be, for example, from 2 to 100 nucleotides, preferably 5 to 20 nucleotides, and more preferably from 8 to 12 nucleotides in length. The initial oligonucleotide frequencies of all possible oligonucleotides in the model sample set can be, for example stored in a look up table, which is accessed as needed. A table defining the model sample set can be constructed, for example, by reference to sample nucleic acid sequences from a previously examined collection of nucleic acids, preferably from a closely related organism, more preferably from the same organism as the nucleic acid sequence under investigation. For example, sample nucleic acid sequences from *Arabidopsis* can be used for a table for investigation of nucleic acid sequences of plants such as soybean, maize, etc. Similarly, sample nucleic acid sequences from a chimpanzee can be used for a table for investigation of nucleic acid sequences of humans. By examining known nucleic acid sequences, model oligonucleotide frequencies in each of the states can be determined. A table can include indefinite or modified nucleotides, or any other nucleotide variations that occur in nucleic acid sequences. Alternatively, it is also possible to use estimation functions in place of such a table of probabilities (*see*, for example, Besemer, J., Borodovsky, M. (1999) *Nucl. Acids Res.*, v.27, pp. 3911-3920, which is herein incorporated by reference in its entirety).

In step 14, the transition probabilities for all nucleotides in the nucleic acid sequence after the initial oligonucleotide in each of the states are determined. The transition probability is the probability of a nucleotide occuring given the oligonucleotide immediately preceding the nucleotide. The transition probability for the first nucleotide transition is set out in equation II:

$$P_f(a_{k+1}|a_1...a_k) = \frac{|a_1...a_{k+1}|_f}{|a_1...a_k|_f} \quad \text{(II)}$$

where k is the oligonucleotide length, a_1 is the first nucleotide in the oligonucleotide, "$a_1...a_k$" is the initial oligonucleotide, a_{k+1} is the nucleotide immediately following a_k, and $f \in \{1+,2+,3+,N+,1-,2-,3-,N-\}$. Equation II determines the transition probability for the first nucleotide following the initial oligonucleotide. After determining the transition probability for

the first nucleotide after the initial oligonucleotide, the transition probabilities are determined sequentially for the remaining nucleotides in the nucleic acid sequence. This means that a transition probability is determined for the second nucleotide after the initial oligonucleotide (a_{k+2}) based on the oligonucleotide beginning at the second position, a_2, and ending at a_{k+1}. The process is repeated until the end of the nucleic acid sequence is reached. For example, if the oligonucleotide length is ten, then a transition probability for nucleotide eleven is determined based on the oligonucleotide comprising nucleotides one through ten. Then, a transition probability for nucleotide twelve is determined based on the oligonucleotide comprising nucleotides two through eleven, and so on, until the last nucleotide in the nucleic acid sequence is reached.

The transition probabilities can be stored in a table, for example. The table can be constructed, for example, by reference to sample nucleic acid sequences from a previously examined portion of nucleic acid, preferably from a closely related organism, more preferably from the same organism as the nucleic acid under investigation. By examining known nucleic acid sequences, model transition probabilities in each of the states can be determined.

In step 16, the probability of the nucleic acid sequence, (S), occurring in each of the states (f) is determined by finding the product of the probability of the initial oligonucleotide and the transition probabilities in each of the states. This step is set forth in equation III for a model with eight states:

$$P_f(S) = P_f(a_1 \ldots a_k) \cdot \prod_{i=1}^{\omega} P_{F(i)}(a_{k+i+1} | a_i \ldots a_{i+k}) \qquad \text{(III)}$$

where the function

$$F(i) = \begin{cases} i \bmod 3 + 1 & \text{if } f = 1^{\pm} \\ (i+1) \bmod 3 + 1 & \text{if } f = 2^{\pm} \\ (i+2) \bmod 3 + 1 & \text{if } f = 3^{\pm} \\ N & \text{if } f = N^{\pm} \end{cases}$$

and ω is the length of the nucleic acid sequence, and "$a_1...a_k$" is the initial oligonucleotide.

In step 18, the probability of each state for the nucleic acid sequence "P(f |S)" is determined given the probability of the nucleic acid sequence, S, in each state. A bias function, $\phi(f)$, is incorporated into the equation to account for known nucleic acid sequence information. This step is set forth in equation IV:

$$P(f|S) = \frac{\phi(f) \cdot P_f \cdot P_f(S)}{\sum_{i \in \{1+,2+,3+,N+,1-,2-,3-,N-\}} \phi(f) \cdot P_i \cdot P_i(S)} \quad \text{(IV)}$$

wherein P_f is $\frac{1}{12}$ for each coding state (1+, 2+, 3+, 1-, 2-, 3-) and $\frac{1}{4}$ for each noncoding state (N+, N-). The bias function is used to modify these default P_f values. By modifying the default values, the investigator can account for known nucleic acid sequence features. For example, if another bioinformatics process has indicated that there is a high probability that a certain portion of a nucleic acid sequence comprises a gene, then it would be advantageous to bias the state probabilities in favor of the coding states. The resulting state probabilities produced by the method will reflect the bias through stronger probabilities of the coding states relative to the noncoding states.

If, for example, the nucleic acid sequence is known to be a coding nucleic acid sequence, the bias function can be defined by equation V:

$$\phi(f) = \begin{cases} 1 & \text{if } f \neq N^{\pm} \\ 0 & \text{if } f = N^{\pm} \end{cases} \quad \text{(V)}$$

Equation V uses a bias of 1 for all coding states, and a bias of 0 for all noncoding states. The net effect will be to cause the probability of the sequence in each noncoding state to drop to

zero, while leaving the probability of the sequence in the coding states unaffected. Application of equation IV then leads to a decrease of the probabilities of the noncoding states to zero, while increasing the probabilities of the coding states.

If the nucleic acid sequence is known to be a noncoding nucleic acid sequence, then the bias function can be defined by equation VI:

$$\phi(f) = \begin{cases} 0 & \text{if } f \neq N^{\pm} \\ 1 & \text{if } f = N^{\pm} \end{cases} \qquad \text{(VI)}$$

Equation VI reverses the effect of equation V. Of course, the bias function does not need to be binary in nature, as is shown in the above two examples, but rather can be defined in any manner that corresponds with known nucleic acid sequence data. A principal feature of this technique is that it can be used to specifically combine gene prediction information from other sources into biasing the results of the state probabilities algorithm shown in Figure 1 (and subsequent gene prediction based thereon).

The resulting values for the probability of each state for the nucleic acid sequence can now be associated with the nucleotide for which state probabilities were being determined.

In a further embodiment of the method shown in Figure 1, the nucleic acid sequence is part of a larger nucleic acid sequence. This embodiment can be applied to any of the methods described herein wherein a nucleic acid sequence is used, including those represented in Figures 1 through 7.

Figure 1 shows the determination of state probabilities for a single nucleotide in a nucleic acid sequence. Oftentimes, however, it will be desirable to determine the state probabilities for more than one nucleotide in a nucleic acid sequence.

Figure 2 represents the application of the method shown in Figure 1 to multiple nucleotides in a nucleic acid sequence. In order to determine the state probabilities for more than one nucleotide, a window is used for each nucleotide that is examined. The nucleotide that is being examined is within the window, and the probability determinations set out in equations I, II, III, and IV are performed for the sequence in the window. The oligonucleotide probabilities

are determined as before for the nucleic acid sequence within the window, probabilities for each of the states are determined for the nucleic acid sequence within the window, and those probabilities are assigned to the nucleotide within the window for which state probabilities are being determined, which, in a preferred embodiment, is the middle nucleotide. Another nucleotide is then examined, with the window shifted or redefined around the new nucleotide, and so on, until the final nucleotide in the nucleic acid sequence for which state probabilities are to be determined is reached.

In steps 22, 24, 26, and 28, probabilities are determined as in steps 12, 14, 16, and 18 respectively, with the window in steps 22, 24, 26, and 28 corresponding to the nucleic acid sequence in steps 12, 14, 16, and 18 respectively for the purposes of those steps. At step 28, the state probabilities for the nucleotide for which state probabilities are being determined are associated with that nucleotide.

In step 30, the algorithm checks to see if the state probabilities for the last nucleotide have just been determined. If yes, flow proceeds to step 32 and ends. If in step 30 the last nucleotide has not been reached, flow proceeds to step 34, where the next nucleotide for which state probabilities are to be determined is designated as the nucleotide to analyze in steps 22, 24, 26, and 28. After step 34, flow returns to steps 22, 24, 26, and 28, where the state probabilities of the designated nucleotide are determined. At step 34 any nucleotide from the remaining nucleotides that have not yet had state probabilities determined can be designated the next nucleotide.

In a preferred embodiment, the first nucleotide to be examined in step 22 is the first nucleotide in a contiguous nucleic acid sequence of nucleotides for which state probabilities are to be determined, each subsequent nucleotide at step 34 is the next nucleotide of the contiguous nucleic acid sequence of nucleotides for which state probabilities are to be determined, and the last nucleotide in step 30 is the last nucleotide in the contiguous nucleic acid sequence of nucleotides for which state probabilities are to be determined.

The window size can be the same or different for each nucleotide, and the nucleotide can be located anywhere within its window. In a preferred embodiment, the window size is the same for each nucleotide in the nucleic acid sequence, and each nucleotide is the middle nucleotide in its own window. In one embodiment, windows are from 3 nucleotides to 1,000 nucleotides in

length, preferably 50 to 200 nucleotides in length, and more preferably from 75 to 125 nucleotides in length.

The result of the process shown in Figure 2 is the association of state probabilities with each individual nucleotide for which state probabilities were determined. In one embodiment, the nucleotides for which state probabilities are to be determined are a contiguous nucleic acid sequence of nucleotides within a longer nucleic acid sequence of nucleotides.

Figures 3 through 7 all utilize probability models to determine state probabilities. Any probability model that allows for determination of the required probabilities in a plurality of states can be used, with use of an inhomogeneous Markov model preferred, and use of the inhomogeneous Markov model described above in reference to Figure 2 especially preferred.

Figure 3 represents one embodiment of a method for determining the coding strand of a nucleic acid sequence. The process determines the state probabilities for each nucleotide in the nucleic acid sequence, sums the positive states for the nucleic acid sequence, and sums the negative states for the nucleic acid sequence. If the sums for the positive states and the negative states are sufficiently different, then the process determines that the state with the greater sum is the coding strand.

In step 38, state probabilities are determined for each nucleotide in the nucleic acid sequence for which the coding strand is being determined. In one embodiment, state probabilities are determined using the inhomogeneous Markov model described above in reference to Figure 2.

In step 40, the probability of each state determined in step 38 for the positive states (1+, 2+, 3+, and N+) for each nucleotide in the nucleic acid sequence for which the coding strand is being determined are summed. That is, the values for the states of noncoding, positive and coding, positive in the first, second, and third reading frames for all nucleotides in the nucleic acid sequence for which the coding strand is being determined are summed. The sum is set to the arbitrary variable X.

In step 42, the values determined in step 38 for the negative states (1-, 2-, 3-, N-) for each nucleotide in the nucleic acid sequence for which the coding strand is being determined are summed. That is, the values for the states of noncoding, negative and coding, negative in the first, second, and third reading frames for all nucleotides in the nucleic acid sequence for which

the coding strand is being determined are summed. The sum is set to the arbitrary variable Y. Steps 40 and 42 can be performed in reverse order.

In step 44, a function of X and Y is used to determine whether the state probabilities indicate sufficient coding on one strand of the nucleic acid sequence. That is, it is determined whether f(X,Y)<T, where T is a defined threshold value. Any function can be used that allows for the desired discrimination. In one embodiment, the function used in step 44 is

$f(X,Y)=\frac{|X-Y|}{(X+Y)}$. When $f(X,Y)=\frac{|X-Y|}{(X+Y)}$, the value of T is about 0.1 to about 0.9, preferably is about 0.25 to about 0.75, and even more preferably is about 0.4 to about 0.6. If in step 44 the function results in a value that is less than the threshold value, T then flow proceeds to step 46, where it is determined that coding is mixed or is not detectable. If in step 44 the function results in a value that is equal to or greater than the threshold value, T, then flow proceeds to step 48.

In step 48, it is determined on which strand coding occurs. A function of X is compared to a function of Y to determine which strand is coding. Any two functions that allow for the proper comparison can be used, including functions that weight one of the two strands. In one embodiment, $f(X)=X$ and $f(Y)=Y$, and the comparison in step 48 simply determines which sum is greater. If in step 48 the function of X is found to be greater than the function of Y, then flow proceeds to step 50 where it is determined that coding is on the positive strand. If in step 48 it is determined that the function of X is not greater than Y, then flow proceeds to step 52, where it is determined that coding is on the negative strand.

In another embodiment of the method represented by Figure 3, steps 44 and 46 can be removed for situations in which it is already known or suspected that coding is present and only on one strand. In this embodiment, flow begins at step 38 and, after executing step 42, flow proceeds directly from step 42 to step 48.

Figure 4 represents one embodiment of a method for determining the extent of an open reading frame (ORF) within a nucleic acid sequence. The process determines the extent of the open reading frame by first determining the state probabilities for each nucleotide in the nucleic acid sequence. Then, beginning from within the nucleic acid sequence, preferably the approximate middle of the nucleic acid sequence, and proceeding toward one end of the nucleic

acid sequence, the process examines each nucleotide in turn and determines whether the nucleotide is sufficiently likely to code. When a sufficient number of nucleotides with an insufficient likelihood of coding are encountered, the process determines that one end of the open reading frame has been found. The process then repeats from the middle to the other end of the nucleic acid sequence in order to find the second end of the open reading frame.

In step 56, the state probabilities of each of the nucleotides in the nucleic acid sequence are determined. As stated above, any probability model that has the correct form of output can be used, with an inhomogeneous Markov model preferred, and the inhomogeneous Markov model described above and represented in Figure 2 most preferred.

In step 58, the coding strand of the nucleic acid sequence is determined and designated "S." Any algorithm or method that can use the state probabilities produced in step 56 can be used, and in a preferred embodiment, the method described above and represented in Figure 3 is used. If coding strand is indeterminate, an error can be returned at this step and processing does not continue. In applications where the coding strand is already known or suspected, step 58 can be omitted from the process, in which case step 56 can flow directly to step 60.

In step 60 an arbitrary variable, L, is set to half of the length of the nucleic acid sequence, S, which designates L the middle nucleotide (determination of the middle for even and odd sequences is done as described above for the middle nucleotide). In an alternative embodiment, L can initially be set to any nucleotide in the nucleic acid sequence. It is preferred, however, to begin with L relatively close to the middle of the putative ORF, because proper resolution of the ends of the ORF is then more likely.

Steps 62, 64, and 66 effectively search through the nucleic acid sequence in a descending direction from L toward the first nucleotide in the nucleic acid sequence for one of the ORF ends. In step 62, the sum of the probabilities of the coding states on the strand S -- that is the set (1+, 2+, and 3+) or the set (1-, 2-, and 3-) depending on whether strand S is the positive or negative strand -- for nucleotide L is determined and compared to threshold value T'. In an alternative embodiment, the probability of all six coding states (1+, 2+, 3+, 1-, 2-, and 3-) can be combined. If the sum of the coding states is greater than or equal to a threshold value, T', and the nucleotide is greater than the first nucleotide in the nucleic acid sequence (that is, L>1), then L is set to L-1 and P, an arbitrary counting variable, is set to L-1. In one embodiment, the value of T is about

0.1 to about 0.9, preferably is about 0.25 to about 0.75, and even more preferably is about 0.4 to about 0.6.

Flow then proceeds to step 64. If the sum of the coding states, as discussed above, is less than T' and P is greater than 1, then P is set to P-1. The effect of the two steps, 62 and 64, is to reduce both L and P at the same rate if the sum of the coding states is greater than or equal to T', or to reduce P but not L if the sum of the states is less than T'.

After step 64, flow proceeds to step 66, where it is determined if L-P>T" or P=1. If L-P>T", wherein T" is a threshold value, then a gap between the last nucleotide (L) with a sufficient sum of coding states and the current nucleotide being examined has increased beyond the threshold value T". T" can be set to any number that allows for the proper gap of noncoding nucleotides. T" should be larger than the maximum expected length of an intron for the nucleic acid sequence. This number will depend in large part on the model sample set being used. If the number for T" is set too low, then a relatively lengthy intron will be sufficient to fix L at the end of an exon that is not at the end of the ORF. If P=1, then the end of the sequence has been reached. In one embodiment, T" is about 10 to about 20,000 nucleotides, preferably about 50 to about 10,000 nucleotides, and more preferably about 500 to about 700 nucleotides.

If neither condition in step 66 is met, then flow returns to step 62 and loops through steps 64 and 66 until one of the conditions in step 66 is met, at which point flow proceeds to step 68. Steps 68, 70, 72, and 74 check for the end of the ORF in the ascending direction, and perform the same function as steps 60, 62, 64, and 66 but in the opposite direction.

In step 68, M is set to the middle nucleotide. As above for L, this value can be altered in alternative embodiments. In step 70, the sum of the coding states, as above, is compared to T', and M is compared to the length of the nucleic acid sequence. If the sum of the coding states of nucleotide M is greater than or equal to T' and M is less than the length of the nucleic acid sequence, then M is set to M+1 and Q is set to M+1. Flow proceeds to step 72, where, if the sum of the coding states is less than T' and Q is less than the length of the nucleic acid sequence, then Q is set to Q+1. Flow proceeds to step 74, where it is determined if Q-M>T", or Q> length of the nucleic acid sequence. If either is true, then flow proceeds to step 76, where the ORF is determined to extend from nucleotide L to nucleotide M. If in step 74 neither condition is true, then flow loops to step 70.

In an alternative embodiment, different threshold values can be used in place of T' and T" for the second loop, which comprises steps 70, 72, and 74. Different threshold values for steps 62, 64, and 66 versus steps 70, 72, and 74 could be desirable if, for example, one end of an ORF was known or suspected to be degraded to some extent.

Figure 5 is a flowchart representing one embodiment of a method for determining the location of deletions and additions within a nucleic acid sequence. The process first determines the state probabilities for each nucleotide in the nucleic acid sequence. Then the process determines whether in the window around a specific nucleotide the most likely state for the nucleic acid sequence on one side of the specific nucleotide is different from the most likely state for the nucleic acid sequence on the other side of the specific nucleotide. If so, the process determines whether a hypothetical insertion or deletion at the specific nucleotide would sufficiently improve the state probabilities of the entire nucleic acid sequence in the window. If so, then an insertion or a deletion is indicated.

In step 78, the state probabilities of each of the nucleotides in the nucleic acid sequence is determined. As stated above, any probability model that has the correct form of output can be used, with an inhomogeneous Markov model preferred, and the inhomogeneous Markov model described above and represented in Figure 2 most preferred.

In step 80, the first nucleotide is designated as "Z," and the size of a window, W, is set. In step 82, the probabilities of each of the states of the nucleotides between Z and the midpoint of the window $Z+\frac{W}{2}$ are averaged, and the state with the greatest average is set to "A" (windows with an even or odd number of nucleotides are treated as above for the middle nucleotide with respect to determination of $\frac{W}{2}$). "A" is effectively the most likely state of the first half of window W.

In step 84, the probabilities of the states of the nucleotides between the midpoint of the window $Z+\frac{W}{2}$ and the end of the window, Z+W, are averaged, and the state with the greatest average is set to B. B is effectively the most likely state of the second half of window W.

In step 86, the most probable states, A and B, are checked to see if they are each a coding state and not the same coding state. If both A and B are coding states and they are not the same

coding state, then flow proceeds to steps 88, 90, and 92, where the nucleotide at $Z+\frac{W}{2}$ is examined further. If, in step 86, A and B are the same coding state, or if one of the two is most probably a noncoding state, then flow proceeds to 96, where it is determined if Z is greater than the length of the nucleic acid sequence minus $\frac{W}{2}$. If so, then flow proceeds to step 98, and the process ends. If, in step 96, Z is not within a distance of $\frac{W}{2}$ of the end of the nucleic acid sequence, then flow proceeds to step 100, where Z is increased by one. Flow then loops to step 82.

If in step 86 if it was determined that both conditions were met, then flow proceeds to steps 88 through 92 to determine if either a deletion or an addition occurred at nucleotide $Z+\frac{W}{2}$.

In step 88, a hypothetical average of state probabilities for state A for the entire window, nucleotides Z to Z+W, for an insertion is determined. The hypothetical average of state probabilities for state A is determined for the window as if the nucleotide at $Z+\frac{W}{2}$ is removed. The probabilities of state A of the nucleotides in W are averaged to obtain the hypothetical average state probabilities for state A for the entire window, and the value is set to N. In step 90, a hypothetical average of state probabilities for state A for the entire window, nucleotides Z to Z+W, for a deletion is calculated similarly. The hypothetical average of state probabilities for state A in step 90 is determined and set to M for the window as if a nucleotide has been added on one side or the other of the nucleotide at $Z+\frac{W}{2}$. By averaging the state probabilities of all of the nucleotides in the window for either an insertion or a deletion, the values of N and M reflect the likelihood that either an insertion or a deletion has taken place. In steps 88 and 90, in an alternative embodiment, state B can be used in place of state A to achieve a similar result.

In step 92, the larger of M and N is compared to the sum of the probabilities of the states indicating coding (1+, 2+, 3+, 1-, 2-, and 3-) of the nucleotide at $Z+\frac{W}{2}$. If in step 92 neither M nor N is greater than the sum of the probabilities of the coding states of the nucleotide at $Z=\frac{W}{2}$,

then it is determined that no insertion or deletion has taken place and flow proceeds to step 96. If in step 92 either M or N is greater than the sum of the probabilities of the coding states of the nucleotide at $Z=\frac{W}{2}$, then it is determined that an insertion or a deletion has taken place, and flow proceeds to step 94.

In step 94, a deletion is indicated if N is greater than M, and an insertion is indicated if N is not greater than M, and flow then proceeds to step 96.

Figure 6 is a flow chart representing one embodiment of a method for determining the location of one or more exons within a nucleic acid sequence and the protein translation of those exons. The process begins by determining the state probabilities for each nucleotide in the nucleic acid sequence, the coding strand, and the extent of the open reading frame. The process then classifies each nucleotide according to its most probable state. Filters, which reclassify nucleotides in a defined manner in order to make local blocks of the nucleic acid sequence consistent, are then applied to the nucleic acid sequence. Regions of the nucleic acid sequence that are in any of classes 1, 2, or 3 are then designated as exons, and the exons are translated. Translation is accomplished by using the universal genetic code to convert the nucleic acid sequence of the designated exons into the corresponding amino acid sequence based on the reading frame of the class. That is, exons in class 1 will be translated in reading frame 1, exons in class two will be translated in reading frame 2, and exons in class 3 will be translated in reading frame 3. The translation is linearly arranged to correspond to the linear arrangement of the exons along the nucleic acid sequence.

In step 102, the state probabilities of each of the nucleotides in the nucleic acid sequence are determined. As stated above, any probability model that has the correct form of output can be used, with an inhomogeneous Markov model preferred, and the inhomogeneous Markov model described above and represented in Figure 2 most preferred. In step 104, the strand and the extent of the open reading frame is determined. Any method for determining the strand and the extent of the ORF that can use the state probabilities generated in step 102 can be used, and in a preferred embodiment, the methods described above and represented in Figures 3 and 4 can be used for such determination.

In step 106, the nucleotides in the nucleic acid sequence are categorized as the highest probability state as determined in step 102. For example, in a model having four states for each nucleic acid strand, each nucleotide is categorized as 1, 2, 3, or N.

In step 108, which is optional, one or more filters are applied to the nucleic acid sequence in order to group adjacent nucleotides by class. Any filter that converts portions of the nucleic acid sequence with inconsistent nucleotide classification to a more homogeneous state can be used. The net effect of the application of one or more filters to the nucleic acid sequence classification in step 104 will be to group adjacent nucleotides and blocks of nucleotides into the same coding classification, thereby making exon and introns more uniform, and exon and intron boundaries more evident.

In step 110, the filtered nucleic acid sequence is analyzed for exons. Any contiguous regions with coding classes of 1, 2, or 3 are determined to be exons. Once each exon has been identified, the exons can be translated using the universal genetic code, and a resulting protein sequence derived.

Figure 7 is a second embodiment of the method described above and represented in Figure 6, with explicit filtering steps detailed therein. In Figure 7, steps 102, 104, 106, and 110 are the same as those described above and shown in Figure 6. In Figure 7, after step 106, steps 112, 114, 116, 118, 120, 122, and 124 are filter steps that are applied to the categorized nucleic acid sequence produced in step 106. The order shown for the filter steps, 112, 114, 116, 118, 120, 122, and 124, can be rearranged to occur in any order in the process, and any combination of the steps can be used, including combinations that omit one or more of the filtering steps.

In step 112, any noncoding nucleotide flanked by two nucleotides with the same class is reclassified into the class of the two flanking nucleotides. For example, 1,N,1 would be converted to 1,1,1.

In step 114, any nucleotide that is flanked by two pairs of adjacent nucleotides all with the same class is reclassified into the class of the flanking nucleotides. For example, 1,1,2,1,1 would be converted to 1,1,1,1,1.

In step 116, any adjacent nucleotide pair having the same class that is flanked by two pairs of adjacent nucleotides all with the same class is reclassified into the class of the flanking nucleotides. For example, 1,1,2,2,1,1 would be converted to 1,1,1,1,1,1.

In step 118, any adjacent nucleotide pair having the same class that is flanked by two nucleotides with the same class is reclassified into the class of the flanking nucleotides. For example, 1,2,2,1 would be converted to 1,1,1,1.

In step 120, any nucleotide flanked by two nucleotides with the same class is reclassified into the class of the flanking nucleotides. For example, 1,2,1 is converted to 1,1,1.

In step 122, any contiguous, noncoding nucleotide region with an insufficient length is reclassified into the class of the flanking coding regions. An insufficient length is any length that is too small to be an intron. This length will be dependent in large part upon the particular nucleic acid sequence under study. In one embodiment, a length of about 10 to 50, preferably about 20 to 40, and more preferably about 25 to 35 nucleotides in length is used. The size of the noncoding nucleotide length required can, in alternative embodiments, be changed as appropriate to better suit examination of the nucleic acid sequence under study. In step 122, the classification of the flanking regions of coding nucleotides can be extended into the noncoding regions an equal amount on either side, an unequal amount on either side, or entirely on one side or the other.

In step 124, any coding region (i.e. a region with nucleotides of classes 1, 2, or 3, comprising more than one nucleotide classification) is reclassified as the most common class in that coding segment.

Flow proceeds to step 110, where the filtered nucleic acid sequence is analyzed for exons. Any contiguous regions with nucleotides of classes 1, 2, or 3 are determined to be exons. Once each exon has been identified, the exons can be translated using the universal genetic code, and a resulting protein sequence derived.

While performing the methods described above in Figures 1-7, windows can sometimes extend past the end of a sequence. Conventional applications that use window-based probability models for multiple nucleotides, such as the windows described above, are limited in their application at the ends of nucleic acid sequences. Since coding probability can be calculated using a window that is centered on each nucleotide of a nucleic acid sequence in turn, a window can extend beyond an end of a sequence. Figure 8a schematically represents a nucleic acid

sequence 200 with a window 204 of length "W." As shown in Figure 8a, the window 204 is empty for the first $\frac{W}{2}$ bases at an end 206 of the sequence 200.

As shown in Figure 8b, the present invention remedies this problem by using the local nucleic acid sequence 216 at the end 206 of the nucleic acid sequence 200 as a source for hypothetical nucleotides added on to the end 206 the nucleic acid sequence 206. As shown in Figure 8c, a copy 218 of the local nucleic acid sequence 216 can be created. As shown in Figure 8d, the copy 218 can then be appended onto the end 206 to form a hypothetical nucleic acid sequence extension. As shown in Figure 8d, the window 204 is now filled with nucleotides from the nucleic acid sequence 200 and the hypothetical nucleic acid sequence extension 218, which allows for probability determination within the window 204. As shown in Figures 8b, 8c, and 8d, the same process can be performed on the other end of the sequence at the same time. Any number of nucleotides can be copied and added in this manner in order to provide the correct size window. In a preferred embodiment, the number of nucleotides copied is a multiple of three. For example, if a 100 nucleotide window is desired for the first nucleotide in the nucleic acid sequence, the first 51 nucleotides of the nucleic acid sequence can be copied to form a hypothetical 51 nucleotide extension. When state probabilities are determined for the first nucleotide, the 51 appended nucleotides are used to fill the first half of the window. The same or different nucleotides can be copied and used in a similar manner for any other nucleotides without a sufficient window. This process can be repeated for the other end of the nucleic acid sequence, of course, as needed. The copied nucleotides can be appended in either orientation on the end of the nucleic acid sequence.

Implementation:

A computer system capable of carrying out the functionality and methods described above is shown in more detail in Figure 9a. A computer system 702 includes one or more processors, such as a processor 704. The processor 704 is connected to a communication bus 706. The computer system 702 also includes a main memory 708, which is preferably random access memory (RAM). Various software embodiments are described in terms of this exemplary computer system. After reading this description, it will become apparent to a person skilled in

the relevant art how to implement the invention using other computer systems and/or computer architectures.

In a further embodiment, shown in Figure 9b, the computer system can also include a secondary memory 710. The secondary memory 710 can include, for example, a hard disk drive 712 and/or a removable storage drive 714, representing a floppy disk drive, a magnetic tape drive, or an optical disk drive, among others. The removable storage drive 714 reads from and/or writes to a removable storage unit 718 in a well known manner. The removable storage unit 718, represents, for example, a floppy disk, magnetic tape, or an optical disk, which is read by and written to by the removable storage drive 714. As will be appreciated, the removable storage unit 718 includes a computer usable storage medium having stored therein computer software and/or data.

In alternative embodiments, the secondary memory 710 may include other similar means for allowing computer programs or other instructions to be loaded into the computer system. Such means can include, for example, a removable storage unit 722 and an interface 720. Examples of such can include a program cartridge and cartridge interface (such as that found in video game devices), a removable memory chip (such as an EPROM, or PROM) and associated socket, and other removable storage units 722 and interfaces 720 which allow software and data to be transferred from the removable storage unit 722 to the computer system.

The computer system can also include a communications interface 724. The communications interface 724 allows software and data to be transferred between the computer system and external devices. Examples of the communications interface 724 can include a modem, a network interface (such as an Ethernet card), a communications port, a PCMCIA slot and card, etc. Software and data transferred via the communications interface 724 are in the form of signals 726 that can be electronic, electromagnetic, optical or other signals capable of being received by the communications interface 724. Signals 726 are provided to communications interface via a channel 728. A channel 728 carries signals 726 in two directions and can be implemented using wire or cable, fiber optics, a phone line, a cellular phone link, an RF link and other communications channels. In one embodiment, the channel is a connection to a network. The network can be any network known in the art, including, but not limited to, LANs, WANs, and the Internet. Nucleic acid sequence data can be stored in remote systems, databases, or

distributed databases, among others, for example GenBank, and transferred to computer system for processing via the network. In a preferred embodiment, nucleic acid sequence data is received through the Internet via the channel 728. Nucleic acid sequences can be input into the system and stored in the main memory 708. Input devices include the communication and storage devices described herein, as well as keyboards, voice input, and other devices for transferring data to a computer system. In a further embodiment, nucleic acid sequences can be generated by an automatic sequencer, for example any that are known in the art, and the implementations described herein can be incorporated within the automatic sequencer device in order to directly use the output of the automatic sequencer.

In this document, the terms "computer program medium" and "computer usable medium" are used to generally refer to media such as the removable storage device 718, a hard disk installed in hard disk drive 712, and signals 726. These computer program products are means for providing software to the computer system.

Computer programs (also called computer control logic) are stored in the main memory 708 and/or the secondary memory 710. Computer programs can also be received via the communications interface 724. Such computer programs, when executed, enable the computer system to perform the features of the present invention as discussed herein. In particular, the computer programs, when executed, enable the processor 704 to perform the features of the present invention. Accordingly, such computer programs represent controllers of the computer system.

In an embodiment where the invention is implemented using software, the software may be stored in a computer program product and loaded into the computer system using the removable storage drive 714, the hard drive 712 or the communications interface 724. The control logic (software), when executed by the processor 704, causes the processor 704 to perform the functions of the invention as described herein.

In another embodiment, the invention is implemented primarily in hardware using, for example, hardware components such as application specific integrated circuits (ASICs). In one embodient incorporating ASIC technology, a self-contained device, which could be hand-held, has integrated circuits specific to perform the methods described above without the need for software. Implementation of such a hardware state machine so as to perform the functions

described herein will be apparent to persons skilled in the relevant art(s). In yet another embodiment, the invention is implemented using a combination of both hardware and software.

The following examples are illustrative only. It is not intended that the present invention be limited to the illustrative embodiments.

EXAMPLE 1

Referring now to Figures 10a, 10b, and 10c, examples of biasing are shown. Figure 10a shows a portion of genomic DNA 300. Aligned with the genomic DNA 300 is an expressed sequence tag (EST) 302. The EST 302 comprises coding regions 304 and noncoding regions 306. In Figure 10b a window 308 of nucleotides is examined. The window 308 is positioned on the genomic DNA 300 that corresponds to a known coding region 304 on the EST 302. The *a priori* probability of coding is said to be 100% over that window 308 and a bias is applied accordingly. In Figure 10c, a different window 310 straddles the intron-exon boundary, and the *a priori* probability of coding is said to be 100% for the nucleotides in the window 310 that correspond to the coding region 304 of the EST 302, while the *a priori* probability of coding is said to be 0% for the nucleotides in the window 310 that correspond to the noncoding region 306 of the EST 302.

Bias is applied to the two different situations shown in Figures 10b and 10c as follows. The general equation for the probability of the sequence $S = a_1...a_w$ of a Markov process of order n is shown in Equation VII:

$$P(a_1...a_\omega) = P(a_1...a_n) \cdot P(a_{n+1}|a_1...a_n) \cdot ...P(a_\omega|a_{\omega-n}...a_{\omega-1}) \quad \text{(VII)}$$

This equation is based on an inhomogeneous Markov model, whereby the initial and transitional probabilities are dependent on the periodic state of the sequence (as in a hidden Markov model with fixed state transition probabilities). In this model, initial and transition probabilities are dependent on the sequence orientation and phase in which the sequence is read relative to the codons in the coding portion of the nucleic acid sequence. Thus, equation VIII is used:

$$P_f(S) = P_f(a_1 \ldots a_n) \cdot \prod_{i=1}^{\omega-n} P_{F(i,\sigma)}(a_{n+i} | a_i \ldots a_{n+i-1}) \quad \text{(VIII)}$$

where, given a state $\sigma \in \{1+, 2+, 3+, N+, 1-, 2-, 3-, N-\}$ representing the possible states for reading the sequence, wherein ...

$$F(i) = \begin{cases} i \bmod 3 + 1 & \text{if } f = 1^{\pm} \\ (i+1) \bmod 3 + 1 & \text{if } f = 2^{+} \\ (i+2) \bmod 3 + 1 & \text{if } f = 3^{\pm} \\ N & \text{if } f = N^{\pm} \end{cases} \quad \text{(IX)}$$

Equation X is used to apply Bayes' rule to determine the probability that the sequence S is in state σ:

$$P(\sigma|S) = \frac{P_\sigma \cdot P_\sigma(S)}{\sum_{i \in \{1^+, 2^+, 3^+, N^+, 1^-, 2^-, 3^-, N^-\}} P_i \cdot P_i(S)} \quad \text{(X)}$$

A bias function is added to equation X in order to allow for biasing of regions of DNA for which coding information is available. The bias function is incorporated in equation XI:

$$P(\sigma|S) = \frac{\phi(\sigma) \cdot P_\sigma \cdot P_\sigma(S)}{\sum_{i \in \{1^+, 2^+, 3^+, N^+, 1^-, 2^-, 3^-, N^-\}} \phi(\sigma) \cdot P_i \cdot P_i(S)} \quad \text{(XI)}$$

Equation XI can be applied to the hypothetical region of DNA shown in the window 308 in figure 10b. Since the entirety of the sequence in the window 308 lies in a coding region (as determined with the EST 302), a bias function $\phi(\sigma)$ can be defined according to equation XII:

$$P(\sigma|S) = \begin{cases} 1 & \text{if } \sigma \in \{1^+, 2^+, 3^+\} \\ 0 & \text{if } \sigma \in \{1^-, 2^-, 3^-, N^+, N^-\} \end{cases} \quad \text{(XII)}$$

which reflects that we know with 100% certainty that the sequence segment must be coding in one of the thee direct reading frames, but that we do not know which. In this case, since $\phi(\sigma) = 0$ where $\sigma \in$ {N+, 1-, 2-, 3-, N-}, equation XII can be written as equation XIII:

$$P(\sigma|S) = \begin{cases} 0 & \text{if } \sigma \in \{1^-, 2^-, 3^-, N^+, N^-\} \\ P_\sigma \cdot P_\sigma(S) \cdot \left[\sum_{i \in \{1^+, 2^+, 3^+\}} P_i \cdot P_i(S) \right]^{-1} & \text{if } \sigma \in \{1^+, 2^+, 3^+\} \end{cases} \quad \text{(XIII)}$$

Because $P_{1+} = P_{2+} = P_{3+}$ (since the EST does not indicate any difference in probability among the three reading frames), equation XIII can be simplified as shown in equation XIV:

$$P(\sigma|S) = \begin{cases} 0 & \text{if } \sigma \in \{1^-, 2^-, 3^-, N^+, N^-\} \\ P_\sigma(S) \cdot \left[\sum_{i \in \{1^+, 2^+, 3^+\}} P_i(S) \right]^{-1} & \text{if } \sigma \in \{1^+, 2^+, 3^+\} \end{cases} \quad \text{(XIV)}$$

The function $\phi(\sigma)$ results in a coding potential (equation XIV) substantially different than the unbiased coding potential function (shown by equation X). In this example, the chosen bias function reduces the probability of the evaluated window 308 to zero in all but the three plus-

strand coding states. This effectively forces the window to be evaluated as coding in one of the positive coding states, while not biasing the probability of those states relative to each other (e.g., $\frac{P_{1+}}{P_{2+}}$ is the same with or without the bias function whereas $\frac{P_{1-}}{P_{1+}}$ may differ).

Figure 10c illustrates a window 310 wherein the evaluated sequence straddles an exon-intron boundary as indicated by the EST 302. A possible function $\phi(\sigma)$ for this situation would be to expand equation XII to equation XIII:

$$P(\sigma|S) = \begin{cases} e & \text{if } \sigma \in \{1^+, 2^+, 3^+\} \\ 1-e & \text{if } \sigma \in \{N^+, N^-\} \\ 0 & \text{if } \sigma \in \{1^-, 2^-, 3^-\} \end{cases} \quad \text{(XIII)}$$

where e represents the fraction of bases in the part of the sequence in the window that lies in the coding region of the DNA 300 as indicated by the coding region 304 of the EST 302. If equation XIII is put into equation IX, equation XIV results:

$$P(\sigma|S) = \begin{cases} 0 & \text{if } \sigma \in \{1^-, 2^-, 3^-\} \\ e \cdot P_\sigma \cdot P_\sigma(S) \cdot \left[\sum_{i \in \{1^+, 2^+, 3^+, N^+, N^-\}} \phi(i) \cdot P_\sigma \cdot P_i(S) \right]^{-1} & \text{if } \sigma \in \{1^+, 2^+, 3^+\} \\ (1-e) \cdot P_\sigma \cdot P_\sigma(S) \cdot \left[\sum_{i \in \{1^+, 2^+, 3^+, N^+, N^-\}} \phi(i) \cdot P_\sigma \cdot P_i(S) \right]^{-1} & \text{if } \sigma \in \{N^+, N^-\} \end{cases} \quad \text{(XIV)}$$

where $P_\sigma = \frac{1}{4}$ for $\sigma \in$ {N+, N-} and $\frac{1}{6}$ for $\sigma \in$ {1+, 2+, 3+} (given the assumption that coding and noncoding are equiprobable events, each coding state is equiprobable with any other coding state, and that both noncoding states are equiprobable, $\frac{1}{4} \times 2 = \frac{1}{2}$ and $\frac{1}{6} \times 3 = \frac{1}{2}$).

EXAMPLE 2

The following example illustrates the computations involved in probability calculations for a sequence with and without a bias applied. The nucleotide sequence GATGACATT is used in this example for clarity and simplicity, but it is understood that longer sequences as indicated above can be used. Further, for this example, a zero order inhomogeneous Markov model is used. In this model, the initial probabilities are all 1 and each event is independent of that which precedes it ($a_1...a_k \rightarrow a_{k+1}$ becomes $N \rightarrow a_1$ because k is zero). Models of higher order can be used, as described above.

Accordingly, the following hypothetical table of probabilities is used:

	Direct (+)			Reverse (-)			
	1+	2+	3+	1-	2-	3-	N±
T	0.13	0.27	0.13	0.10	0.25	0.21	0.20
C	0.28	0.26	0.39	0.39	0.21	0.38	0.30
A	0.21	0.26	0.09	0.13	0.27	0.13	0.21
G	0.38	0.21	0.39	0.38	0.26	0.28	0.29

Without a bias function $\phi(\sigma)$ to incorporate known information in the calculations, $P(S|\sigma)$ can be calculated for the zero order case for the sequence GATGACATT according to equations XV through XXI.

$$
\begin{aligned}
P(\texttt{GATGACATT}|1^+) &= P(N)\cdot P_{1+}(G|N)\cdot P_{2+}(A|N)\cdot P_{3+}(T|N)\cdot \\
&\quad P_{1+}(G|N)\cdot P_{2+}(A|N)\cdot P_{3+}(C|N)\cdot \\
&\quad P_{1+}(A|N)\cdot P_{2+}(T|N)\cdot P_{3+}(T|N) \\
&= P_{1+}(G)\cdot P_{2+}(A)\cdot P_{3+}(T)\cdot \\
&\quad P_{1+}(G)\cdot P_{2+}(A)\cdot P_{3+}(C)\cdot \\
&\quad P_{1+}(A)\cdot P_{2+}(T)\cdot P_{3+}(T) \\
&= 0.38\times 0.26\times 0.13\times 0.38\times 0.26\times \\
&\quad 0.39\times 0.21\times 0.27\times 0.13 \\
&= 3.6479448\times 10^{-6}
\end{aligned}
\tag{XV}
$$

$$
\begin{aligned}
P(\texttt{GATGACATT}|2^+) &= P_{2+}(G)\cdot P_{3+}(A)\cdot P_{1+}(T)\cdot \\
&\quad P_{2+}(G)\cdot P_{3+}(A)\cdot P_{1+}(C)\cdot \\
&\quad P_{2+}(A)\cdot P_{3+}(T)\cdot P_{1+}(T) \\
&= 0.21\times 0.09\times 0.13\times 0.21\times 0.09\times \\
&\quad 0.28\times 0.26\times 0.13\times 0.13 \\
&= 5.71332739\times 10^{-8}
\end{aligned}
\tag{XVI}
$$

$$
\begin{aligned}
P(\texttt{GATGACATT}|3^+) &= P_{3+}(G)\cdot P_{1+}(A)\cdot P_{2+}(T)\cdot \\
&\quad P_{3+}(G)\cdot P_{1+}(A)\cdot P_{2+}(C)\cdot \\
&\quad P_{3+}(A)\cdot P_{1+}(T)\cdot P_{2+}(T) \\
&= 0.39\times 0.21\times 0.27\times 0.39\times 0.21\times \\
&\quad 0.26\times 0.09\times 0.13\times 0.27 \\
&= 1.4874917\times 10^{-6}
\end{aligned}
\tag{XVII}
$$

$$
\begin{aligned}
P(\texttt{GATGACATT}|1^-) &= P_{1-}(G)\cdot P_{2-}(A)\cdot P_{3-}(T)\cdot \\
&\quad P_{1-}(G)\cdot P_{2-}(A)\cdot P_{3-}(C)\cdot \\
&\quad P_{1-}(A)\cdot P_{2-}(T)\cdot P_{3-}(T) \\
&= 0.38\times 0.27\times 0.21\times 0.38\times 0.27\times \\
&\quad 0.38\times 0.13\times 0.25\times 0.21 \\
&= 5.7332419\times 10^{-6}
\end{aligned}
\tag{XVIII}
$$

$$
\begin{aligned}
P(\texttt{GATGACATT}|2^-) &= P_{2^-}(G)\cdot P_{3^-}(A)\cdot P_{1^-}(T)\cdot \\
&\quad P_{2^-}(G)\cdot P_{3^-}(A)\cdot P_{1^-}(C)\cdot \\
&\quad P_{2^-}(A)\cdot P_{3^-}(T)\cdot P_{1^-}(T) \\
&= 0.26\times 0.13\times 0.10\times 0.26\times 0.13\times \\
&\quad 0.39\times 0.27\times 0.21\times 0.10 \\
&= 2.5262776\times 10^{-7}
\end{aligned}
$$

(XIX)

$$
\begin{aligned}
P(\texttt{GATGACATT}|3\ ) &= P_{3^-}(G)\cdot P_{1^-}(A)\cdot P_{2^-}(T)\cdot \\
&\quad P_{3^-}(G)\cdot P_{1^-}(A)\cdot P_{2^-}(C)\cdot \\
&\quad P_{3^-}(A)\cdot P_{1^-}(T)\cdot P_{2^-}(T) \\
&= 0.28\times 0.13\times 0.25\times 0.28\times 0.13\times \\
&\quad 0.21\times 0.13\times 0.10\times 0.25 \\
&= 2.2607130\times 10^{-7}
\end{aligned}
$$

(XX)

$$
\begin{aligned}
P(\texttt{GATGACATT}|N) &= P_N(G)\cdot P_N(A)\cdot P_N(T)\cdot \\
&\quad P_N(G)\cdot P_N(A)\cdot P_N(C)\cdot \\
&\quad P_N(A)\cdot P_N(T)\cdot P_N(T) \\
&= 0.29\times 0.21\times 0.20\times 0.29\times 0.21\times \\
&\quad 0.30\times 0.21\times 0.20\times 0.20 \\
&= 1.8692402\times 10^{-6}
\end{aligned}
$$

(XXI)

Given the values of P(S|σ), we can determine the probability that the given sequence segment is in state σ, P(σ|S) using equation XXII (Bayes' Rules):

$$P(\sigma|S) = \frac{P(\sigma)\cdot P(S|\sigma)}{\sum_i [P(i)\cdot P(S|i)]}$$

(XXII)

Equations XXIII through XXIX show the calculations for each of the states.

$$P(1^+|S) = \frac{P(1^+)\cdot P(S|1^+)}{\sum_i [P(i)\cdot P(S|i)]}$$
$$= \frac{\frac{1}{12}(3.6479448\times10^{-6})}{\frac{1}{12}(3.6479448\times10^{-6})+\ldots+\frac{1}{2}(1.8692402\times10^{-6})}$$
$$= \frac{3.0399540\times10^{-7}}{1.1060761\times10^{-6}}$$
$$= 0.27484131$$

(XXIII)

$$P(2^+|S) = \frac{4.7611061\times10^{-9}}{1.1060761\times10^{-6}}$$
$$= 0.004304501$$

(XXIV)

$$P(3^+|S) = \frac{1.12395764\times10^{-7}}{1.1060761\times10^{-6}}$$
$$= 0.11156173$$

(XXV)

$$P(1^-|S) = \frac{4.7777016\times10^{-7}}{1.1060761\times10^{-6}}$$
$$= 0.43195053$$

(XXVI)

$$P(2^-|S) = \frac{2.1052313\times10^{-8}}{1.1060761\times10^{-6}}$$
$$= 0.019033331$$

(XXVII)

$$P(3^-|S) = \frac{1.8839275\times10^{-8}}{1.1060761\times10^{-6}}$$
$$= 0.017032531$$

(XXVIII)

$$P(N|S) = \frac{1.557002\times10^{-7}}{1.1060761\times10^{-6}} = 0.14076807$$

(XXIX)

The coding probability function indicates a 43% probability that the sequence is coding in the first reading frame of the reverse-complement strand (-) of the sequence provided, based on the zero order inhomogeneous Markov model used. While the most probable state, it is also true that there is a greater probability (57%) that the sequence is not in that state.

An investigator can apply the bias function method to impose a bias based on prior knowledge of sequence features, such as an EST alignment to the subject sequence, or homology to a previously characterized sequence. For example, given an EST alignment to the subject sequence that implies the sequence is coding on the positive strand, a bias function can be defined that summarizes that observation. Equation XXX is one example of such a function:

$$\phi(\sigma) = \begin{cases} 0.95 & \text{if } \sigma \in \{1^+, 2^+, 3^+\} \\ 0.05 & \text{if } \sigma \notin \{1^+, 2^+, 3^+\} \end{cases}$$

(XXX)

This bias function does not exclude the possibility that the sequence is noncoding or coding on the reverse complement strand, although it does effectively bias the *a priori* probability that the sequence is coding in one of the forward three reading frames. The function above states that the three forward coding states are 19-fold (0.95/0.05) more probable than the other states, which is an assertion by the investigator that he is confident that the EST alignment is correct in indicating that the sequence is coding on that strand.

Given the bias function defined above, the values for P'(S|σ) are determined as before for the unbiased case. To calculate P'(σ|S), however, equation XXXI is used:

$$P'(\sigma|S) = \frac{\phi(\sigma) \cdot P(\sigma) \cdot P(S|\sigma)}{\sum_i [\phi(i) \cdot P(i) \cdot P(S|i)]}$$

(XXXI)

The equations to determine P'(σ|S) for each state are shown in equations XXXII through XXXVIII:

$$\begin{aligned} P'(1^+|S) &= \frac{\phi(1^+) \cdot P(1^+) \cdot P(S|1^+)}{\sum_i [\phi(i) \cdot P(i) \cdot P(S|i)]} \\ &= \frac{0.95 \cdot \frac{1}{12}(3.6479448 \times 10^{-6})}{0.95 \cdot \frac{1}{12}(3.6479448 \times 10^{-6}) + \ldots + 0.05 \cdot \frac{1}{2}(1.8692402 \times 10^{-6})} \\ &= \frac{2.8879563 \times 10^{-7}}{4.4399294 \times 10^{-7}} \\ &= 0.65045095 \end{aligned}$$

(XXXII)

$$\begin{aligned} P'(2^1|S) &= 0.95 \frac{\frac{1}{12} \cdot P(S|2^+)}{4.4399294 \times 10^{-7}} \\ &= 0.010187213 \end{aligned}$$

(XXXIII)

$$\begin{aligned} P'(3^1|S) &= 0.95 \frac{\frac{1}{12} \cdot P(S|3^+)}{4.4399294 \times 10^{-7}} \\ &= 0.2652289 \end{aligned}$$

(XXXIV)

$$\begin{aligned} P'(1^-|S) &= 0.05 \frac{\frac{1}{12} \cdot P(S|1^-)}{4.4399294 \times 10^{-7}} \\ &= 0.05380379 \end{aligned}$$

(XXXV)

$$P'(2^-|S) = 0.05\frac{\frac{1}{12}\cdot P(S|2^-)}{4.4399294\times10^{-7}} = 0.0023707938$$

(XXXVI)

$$P'(3^-|S) = 0.05\frac{\frac{1}{12}\cdot P(S|3^-)}{4.4399294\times10^{-7}} = 0.00042392676$$

(XXXVII)

$$P'(N|S) = 0.05\frac{\frac{1}{2}\cdot P(S|N)}{4.4399294\times10^{-7}} = 0.0017534085$$

(XXXVIII)

Given the bias function ø(σ), the resulting coding potential calculation indicates a 65% probability that the sequence is coding in the first reading frame on the forward strand. The result represents the coding probability given the assumptions of the investigator stated as the bias function.

EXAMPLE 3

The following is a copy of the output of a program implementing the method described above with and without a bias function. The following sequence is a genomic sample from the organism *Arabidopsis thaliana, landsberg*.

TACTCAAAAATATATTCCATGCTTAATTAGGCCGGATTCGCGGTGACGATGCACCAAGAGCGGTTTTTCCGA
GCATTGTAGGCCGTCCTCGCCACACCGGTGTGATGGTTGGGATGGGACAAAAGGATGCTTATGTTGGAGACGAGGCTC
AATCAAAACGTGGTATCTTGACTCTGAAGTACCCAATTGAGCATGGAATTGTTAATAATTGGGATGACATGGAGAAGA
TTTGGCATCACACTTTCTACAATGAGCTTCGTGTTGCCCCTGAAGAACATCCGGTTCTCTTGACCGAAGCTCCTCTCA
ATCCGAAAGCTAACCGTGAGAAGATGACTCAGATCATGTTTGAGACATTCAATACTCCTGCTATGTATGTTGCCATTC
AAGCTGTTCTCTCACTCTATGCCAGTGGCCGTACTACTGGTCAGTACATTACTACATTCTTTTTATACCGTTTGGTTG
AAATAAAATTCGGTTTGGTTCGATTCGAGTTTGCTCTCATTATTTTTATTTTGTTGGTTAGGTATTGTTTTGGACTCC
GGAGATGGTGTGAGCCACACGGTACCAATCTACGAGGGTTATGCACTTCCACACGCAATCCTGCGTCTTGATCTTGCA
GGTCGTGACCTAACCGACCACCTTATGAAAATCCTGACAGAGCGTGGTTACTCTTTCACCACAACTGCTGAGCGTGAG

ATTGTTAGAGACATGAAGGAGAAGCTCTCTTACATTGCCTTGGACTTTGAACAAGAGCTCGAGACTTCCAAAACAAGC
TCATCCGTTGAGAAGAGCTTCGAGCTGCCAGACGGTCAAGTGATCACCATCGGGGCAGAGCGTTTCCGATGCCCTGAA
GTTCTGTTTCAGCCATCGATGATCGGAATGGAAAATCCGGGAATTCATGAAACTACTTACAACTCAATCATGAAATGT
GATGTGGATATCAGGAAGGATCTTTATGGAAACATTGTGCTTAGTGGTGGCACCACAATGTTCGATGGGATTGGTGAT
AGGATGAGTAAAGAGATCACAGCGTTGGCTCCAAGCAGTATGAAGATCAAAGTGGTGGCTCCACCGGAAAGGAAGTAC
AGTGTCTGGATCGGTGGCTCTATCTTGGCTTCCCTCAGTACTTTCCAGCAGGTAAATTACTTACTATACTTAATACAT
AAAGTCTATTAGTGATTTGATGTATAAAGTGTTACAAAAATGTGTTCCAAATTTGCAGATGTGGATTGCGAAAGCGGA
GTATGATGAATCTGGACCGTCAATCGTCCACAGGAAGTGCTTCTGATCAAAAGTCACCAAGTAAAACAAGAGCGGTAA
AAATTTTGATATCAGTTTTTCACCCTGAAGCCAGTTGCTATAATTACTCACAACTTCTCTATTTGTGTTCTTTTATTC
TTGTCCCTCGTTGTTCATTTTAATCTCTTTTTTGCAACAAAGCAACTTAAAAAAACAGAGCAGTCATTAACAGAATGT
TATTATTATATATATGTATACATATTAGTATACACCCATTATTTCATTAAAACATTTATCATATAAGGATAGGATTCT
ATACATCGATATATTTATTTTGTTGACACTATTCAGCACATGCTTATGTCTTATCTTGTTAGTATATGTAACCAAAGA
CAAATAATAGATGCTACAAATTGTTTTCTTTGAAGCAAAAATTTCAATCTTAAAATTGTTTTTTTCCAGGTTACACAA
AAAAAACTTGTAGTTTGTAAATTTTCTATACAATTTTGGGGATCTCAACAAGAACATGAACTTCAACTTCTAGTCATA
TGACGACCTGAGTCTGCGCGGCTGTGAATCTCTTTGCTGCAGTAAATGTTTACAAGTGGTGTGTAAATTGGTACTGAT
TCAAAAGCTTTAAGAAATCTACACATTTCGTGAAATTATTTAGCAGACTTGATATTAAAAATCTAGGATAAAATGACT
ATCCAAAGACAAATAGGACTGTTTCACATGTTCCCCTGATTCTTGTAGCTCATAACTCATCAGCAGTTAACTTTTCTA
CCTCATACACGCTCGCAATNCGTTTGGAATTATCAGCTNTAATTTTTCTAATTCTTTGGAAATTATTAGCAGCTCGAT
CAAATGGGGCATGGCTTCTTCTTCTATCTGCAACTCATCTAAACTTTCCATGAAGAAACAAAGCT (SEQ. ID.
NO. 1)

The sequence below is the same *Arabidopsis* sequence after coding probabilities have been determined without a bias, the coding strand has been determined, and each nucleotide has been classified in its most probable state of the four on the coding strand (dashes represent the state of noncoding).

```
   1: ------------------------------------------------------------1
  61: 1111111111113133333333333333313333333333333333333333333333
 121: 3333233333333333333333333331333333333333333333333333333333
 181: 3333333333333333333333333333333133331333333333333133333333
 241: 3333333331331333333313333333333333333333333333333333333133
 301: 3333333333333331333333333333333331333333333333333333333333
 361: 333-33333-333333-3333333333333333-3333333333333333333333333
 421: 333333333333--3--3---333333333-33--------------------------
 481: ------------------------------------------------11---11-1-
 541: -1111111111111111111111111111111111111111111111111111111-111
 601: 1111111111111111111111111111111111111111111111111111111-1111
 661: 1111111111-111-11111111111111-1111111111111111111111111111111
 721: 1111111111111-11111-11111111-1111111111111111111111111111111
 781: 1111111111111111111111111111111111111111111111111111111111111
 841: 1111111111111111111111111111111111111111111111111111111-1111
 901: 1111111111111111-1111111111111111111111111111-1111111111111
 961: 1111111111111111111111111111111111111111111111111111111111111
1021: 1111111111111111111111111111111111111111111111111111111111111
1081: 1111111111111131111111111111131----------------------------
1141: ------------------------------------------------------------
1201: ------222-2222222222-22-222-222222-333333333333333333333333
1261: 3333333333333333--33-3--3--3----33-33333333-333------------
1321: ---333--3---------------------------------------------------
1381: ------------------------------------------------------------
```

```
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: --------------------1--1--1-------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: ----------3---33-3-------333-3--------------------------3
2041: 3-3133-33-33-3----13-22222-222222-2222222222222-2----------2
2101: --22--------2222-12222222222222222212222222222222222222222222
2161: 22222
```

The classifications are now filtered. First, simple gaps are filled (XYX are reclassified as XXX):

```
   1: ------------------------------------------------------------1
  61: 11111111111131333333333333333313333333333333333333333333333
 121: 33332333333333333333333333313333333333333333333333333333333
 181: 33333333333333333333333333333331333331333333333333133333
 241: 33333333313313333333313333333333333333333333333333333133
 301: 33333333333333313333333333333333313333333333333333333333
 361: 33333333333333333333333333333333333333333333333333333333
 421: 333333333333--3--3---333333333333--------------------------
 481: ------------------------------------------------11---1111-
 541: -11111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111
1081: 1111111111111131111111111111131----------------------------
1141: ------------------------------------------------------------
1201: ------222222222222222222222222-333333333333333333333333
1261: 3333333333333333--3333--3--3----333333333333333----------
1321: ---333--3---------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: --------------------1--1--1-------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: ----------3---3333-------33333--------------------------3
2041: 33313333333333----13-22222222222222222222222222----------2
2101: --22--------2222-12222222222222222212222222222222222222222222
```

```
2161: 22222
```

Next, XXYXX gaps are reclassified as XXXXX:

```
   1: ------------------------------------------------------------1
  61: 1111111111113133333333333333333333333333333333333333333333
 121: 3333333333333333333333333333333333333333333333333333333333
 181: 3333333333333333333333333333333333333333333333333333333333
 241: 3333333333333333333333333333333333333333333333333333333333
 301: 3333333333333333333333333333333333333333333333333333333333
 361: 3333333333333333333333333333333333333333333333333333333333
 421: 333333333333---------333333333333--------------------------
 481: ------------------------------------------------11---1111-
 541: -1111111111111111111111111111111111111111111111111111111111
 601: 1111111111111111111111111111111111111111111111111111111111
 661: 1111111111111111111111111111111111111111111111111111111111
 721: 1111111111111111111111111111111111111111111111111111111111
 781: 1111111111111111111111111111111111111111111111111111111111
 841: 1111111111111111111111111111111111111111111111111111111111
 901: 1111111111111111111111111111111111111111111111111111111111
 961: 1111111111111111111111111111111111111111111111111111111111
1021: 1111111111111111111111111111111111111111111111111111111111
1081: 11111111111111111111111111131----------------------------
1141: ------------------------------------------------------------
1201: ------222222222222222222222222-333333333333333333333333
1261: 3333333333333333--3333----------333333333333333-----------
1321: ---333-----------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------3333-------33333---------------------------3
2041: 33333333333333----13-22222222222222222222222222-----------
2101: --22--------2222-12222222222222222222222222222222222222222
2161: 22222
```

Next, XXYYXX gaps are reclassified as XXXXXX:

```
   1: ------------------------------------------------------------1
  61: 1111111111113133333333333333333333333333333333333333333333
 121: 3333333333333333333333333333333333333333333333333333333333
 181: 3333333333333333333333333333333333333333333333333333333333
 241: 3333333333333333333333333333333333333333333333333333333333
 301: 3333333333333333333333333333333333333333333333333333333333
 361: 3333333333333333333333333333333333333333333333333333333333
 421: 333333333333---------333333333333--------------------------
 481: ------------------------------------------------------11111
```

```
 541: 11111111111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111111
1081: 1111111111111111111111111131----------------------------
1141: ------------------------------------------------------------
1201: ------222222222222222222222222-333333333333333333333333
1261: 3333333333333333333----------333333333333333-------------
1321: ---333-------------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------3333-------33333----------------------------3
2041: 33333333333333----13-22222222222222222222222222-----------
2101: ------------222222222222222222222222222222222222222222222
2161: 22222
```

Next, XYYX gaps are reclassified as XXXX:

```
   1: ------------------------------------------------------------1
  61: 11111111111131333333333333333333333333333333333333333333333
 121: 33333333333333333333333333333333333333333333333333333333333
 181: 33333333333333333333333333333333333333333333333333333333333
 241: 33333333333333333333333333333333333333333333333333333333333
 301: 33333333333333333333333333333333333333333333333333333333333
 361: 33333333333333333333333333333333333333333333333333333333333
 421: 333333333333---------333333333333----------------------------
 481: ------------------------------------------------------11111
 541: 11111111111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111111
1081: 1111111111111111111111111131----------------------------
1141: ------------------------------------------------------------
1201: ------222222222222222222222222-333333333333333333333333
1261: 3333333333333333333----------333333333333333-------------
```

```
1321: ---333-----------------------------------------------------------
1381: -----------------------------------------------------------------
1441: -----------------------------------------------------------------
1501: -----------------------------------------------------------------
1561: -----------------------------------------------------------------
1621: -----------------------------------------------------------------
1681: -----------------------------------------------------------------
1741: -----------------------------------------------------------------
1801: -----------------------------------------------------------------
1861: -----------------------------------------------------------------
1921: -----------------------------------------------------------------
1981: --------------3333-------33333----------------------------3
2041: 33333333333333----13-22222222222222222222222222-----------
2101: ------------2222222222222222222222222222222222222222222222
2161: 22222
```

Next, XYX gaps are reclassified as XXX:

```
   1: -----------------------------------------------------------1
  61: 11111111111113333333333333333333333333333333333333333333
 121: 33333333333333333333333333333333333333333333333333333333
 181: 33333333333333333333333333333333333333333333333333333333
 241: 33333333333333333333333333333333333333333333333333333333
 301: 33333333333333333333333333333333333333333333333333333333
 361: 33333333333333333333333333333333333333333333333333333333
 421: 333333333333---------333333333333-------------------------
 481: -----------------------------------------------------11111
 541: 11111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111
1081: 1111111111111111111111111111----------------------------
1141: -----------------------------------------------------------
1201: ------222222222222222222222222-333333333333333333333333
1261: 3333333333333333333----------333333333333333-------------
1321: ---333-----------------------------------------------------------
1381: -----------------------------------------------------------------
1441: -----------------------------------------------------------------
1501: -----------------------------------------------------------------
1561: -----------------------------------------------------------------
1621: -----------------------------------------------------------------
1681: -----------------------------------------------------------------
1741: -----------------------------------------------------------------
1801: -----------------------------------------------------------------
1861: -----------------------------------------------------------------
1921: -----------------------------------------------------------------
1981: --------------3333-------33333----------------------------3
2041: 33333333333333----13-22222222222222222222222222-----------
```

```
2101: ------------2222222222222222222222222222222222222222222222
2161: 22222
```

Next, regions between coding regions that are not introns are reclassified according to the adjacent sequences:

```
   1: ----------------------------------------------------------1
  61: 1111111111111133333333333333333333333333333333333333333333
 121: 3333333333333333333333333333333333333333333333333333333333
 181: 3333333333333333333333333333333333333333333333333333333333
 241: 3333333333333333333333333333333333333333333333333333333333
 301: 3333333333333333333333333333333333333333333333333333333333
 361: 3333333333333333333333333333333333333333333333333333333333
 421: 333333333333333333333333333333----------------------------
 481: ----------------------------------------------------11111
 541: 1111111111111111111111111111111111111111111111111111111111
 601: 1111111111111111111111111111111111111111111111111111111111
 661: 1111111111111111111111111111111111111111111111111111111111
 721: 1111111111111111111111111111111111111111111111111111111111
 781: 1111111111111111111111111111111111111111111111111111111111
 841: 1111111111111111111111111111111111111111111111111111111111
 901: 1111111111111111111111111111111111111111111111111111111111
 961: 1111111111111111111111111111111111111111111111111111111111
1021: 1111111111111111111111111111111111111111111111111111111111
1081: 11111111111111111111111111111----------------------------
1141: ------------------------------------------------------------
1201: ------222222222222222222222222223333333333333333333333333
1261: 3333333333333333333333333333333333333333333333333333333333
1321: 333333------------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------333333333333333333333333333333333333333333
2041: 33333333333333311322222222222222222222222222222222222222
2101: 22222222222222222222222222222222222222222222222222222222
2161: 22222
```

Next, the sequence is checked for frameshifts and reclassified accordingly:

```
   1: ----------------------------------------------------------1
  61: 1111111111111111111111111111111111111111111111111111111111
 121: 1111111111111111111111111111111111111111111111111111111111
 181: 1111111111111111111111111111111111111111111111111111111111
 241: 1111111111111111111111111111111111111111133333333333333333
 301: 3333333333333333333333333333333333333333333333333333333333
 361: 3333333333333333333333333333333333333333333333333333333333
```

```
 421: 333333333333333333333333333333333----------------------------
 481: ----------------------------------------------------------11111
 541: 11111111111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111111
1081: 1111111111111111111111111111111----------------------------
1141: ------------------------------------------------------------
1201: ------222222222222222222222222222222222222233333333333333
1261: 333333333333333333333333333333333333333333333333333333333333
1321: 333333------------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------333333333333333333333333333333333333333333333
2041: 333333333333333333333333333333322222222222222222222222222222
2101: 222222222222222222222222222222222222222222222222222222222222
2161: 22222
```

Finally, the sequence is translated according to each class in each coding region, where an "x" indicates a stop codon:

```
   1 : XRFFRALxAVLATPVxWLGWDKRMLMLETRLNQNVVSxLxSTQLSMELLIIGMTWRRFGI
  61 : TLSTMSFVLPLKNIRXLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRT
 121 : TGQYITTFFLYRXSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDHLMKILTERGYSFT
 181 : TTAEREIVRDMKEKLSYIALDFEQELETSKTSSSVEKSFELPDGQVITIGAERFRCPEVL
 241 : FQPSMIGMENPGIHETTYNSIMKCDVDIRKDLYGNIVLSGGTTMFDGIGDRMSKEITALA
 301 : PSSMKIKVVAPPERKYSVWIGGSIXVPNLQMWIAKAEYXNLDRQSSTGSASDQKSPSKTR
 361 : AVKILXNSSAVNFSTSYTLAIRLELSALIFLISLEIISSSIKWGMASSSICNSSKLSMKK
 421 : QSX (SEQ. ID. NO. 2)
```

The following sequence is the same *Arabidopsis* sequence used above, but with an applied bias. Two bias functions are given by equations XXXIX and XL:

$$\phi_1(\sigma) = \begin{cases} 0.95 & \text{if } \sigma \in \{1^+, 2^+, 3^+, 1^-, 2^-, 3^-\} \\ 0.05 & \text{if } \sigma = N \end{cases}$$

(XXXIX)

$$\phi_2(\sigma) = \begin{cases} 0.05 & \text{if } \sigma \in \{1^+, 2^+, 3^+, 1^-, 2\ \ , 3^-\} \\ 0.95 & \text{if } \sigma = N \end{cases}$$

(XL)

where ϕ_1 is applied to a range of the DNA to which an EST has been associated, while ϕ_2 is applied to a range of the DNA to which a gap (or intron) in the EST has been associated. Specifically, ϕ_1 is applied to nucleotides 1093 through 1137 and 1219 through 1291, while ϕ_2 is applied to nucleotides 1138 through 1218. The probabilities are calculated with the bias, the coding strand is determined, and each nucleotide is classified as the most likely state. The resulting sequence is depicted below.

```
   1: ------------------------------------------------------------1
  61: 1111111111113133333333333333331333333333333333333333333333
 121: 3333233333333333333333333331333333333333333333333333333333
 181: 3333333333333333333333333333333313333313333333333333133333
 241: 3333333331331333333331333333333333333333333333333333333133
 301: 3333333333333331333333333333333331333333333333333333333333
 361: 333-33333-333333-333333333333333-333333333333333333333333333
 421: 333333333333--3--3---333333333-33--------------------------
 481: ------------------------------------------------11---11-1-
 541: -11111111111111111111111111111111111111111111111111111111-111
 601: 11111111111111111111111111111111111111111111111111111111-1111
 661: 1111111111-111-11111111111111-11111111111111111111111111111111
 721: 1111111111111-11111-11111111-11111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111-1111
 901: 1111111111111111-11111111111111111111111111-1111111111111
 961: 11111111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111111
1081: 1111111111111131111111111111311111111-1--------------------
1141: ------------------------------------------------------------
1201: ------------------2212212221222222133333333333333333333333333
1261: 333333333333333333333333333333-33-33333333-333-------------
1321: ---333--3-------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
```

```
1741: ------------------------------------------------------------
1801: --------------------1--1--1-------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: ----------3---33-3-------333-3--------------------------3
2041: 3-3133-33-33-3----13-22222-222222-2222222222222-2----------2
2101: --22--------2222-12222222222222222212222222222222222222222222
2161: 22222
```

Filtering steps are then applied as before: XYX to XXX:

```
   1: ------------------------------------------------------------1
  61: 11111111111131333333333333333313333333333333333333333333333
 121: 33332333333333333333333333313333333333333333333333333333333
 181: 33333333333333333333333333333313333313333333333333133333
 241: 33333333313313333333313333333333333333333333333333333333133
 301: 33333333333333313333333333333333313333333333333333333333333
 361: 33333333333333333333333333333333333333333333333333333333333
 421: 333333333333--3--3---333333333333-------------------------
 481: ------------------------------------------------11---1111-
 541: -11111111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111
1081: 1111111111111131111111111111131111111111----------------------
1141: ------------------------------------------------------------
1201: ------------------2212212221222222133333333333333333333333
1261: 333333333333333333333333333333333333333333333----------------
1321: ---333--3-------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: --------------------1--1--1-------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: ----------3---3333-------33333--------------------------3
2041: 33313333333333----13-22222222222222222222222222----------2
2101: --22--------2222-12222222222222222212222222222222222222222222
2161: 22222
```

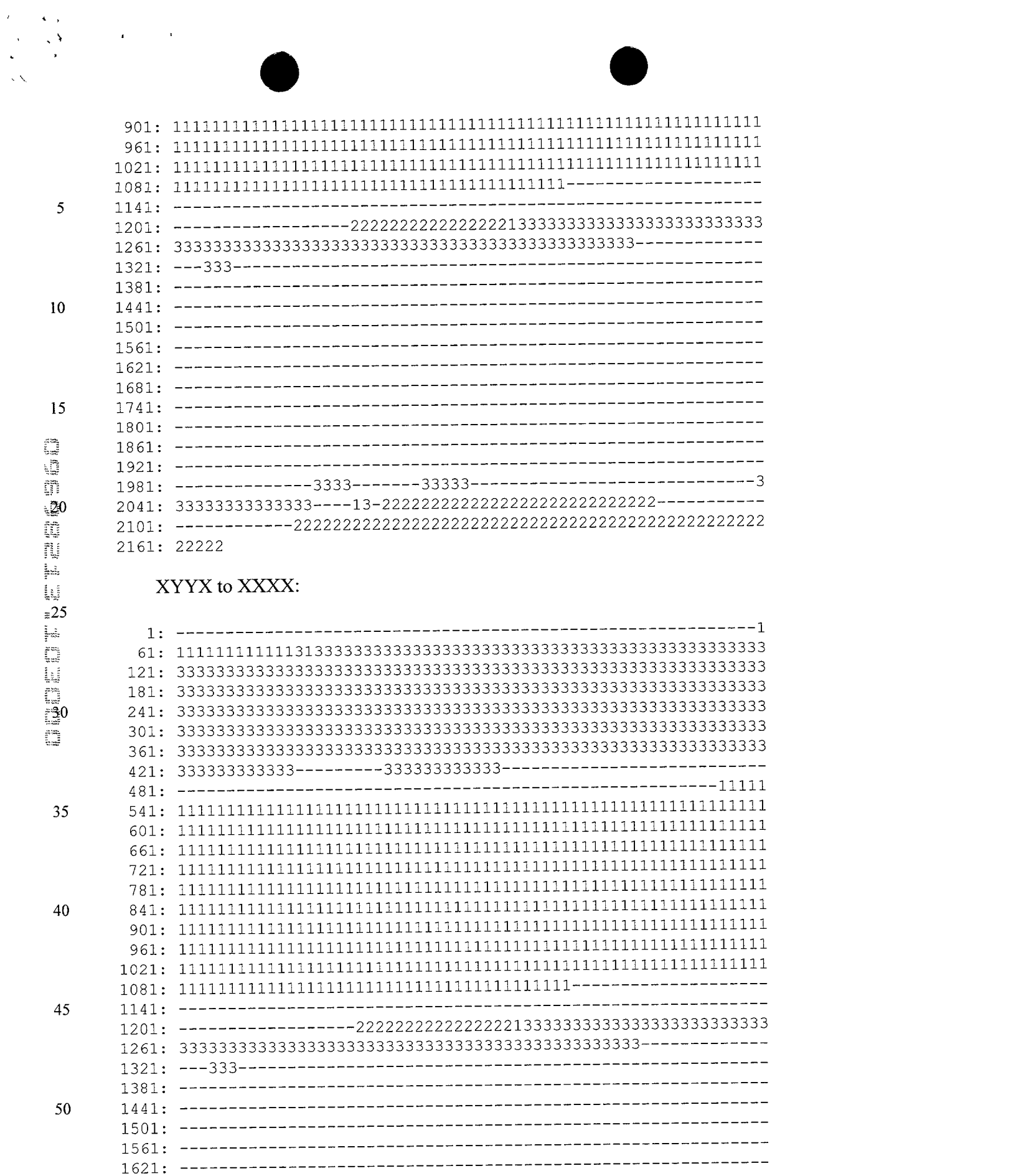
XXYXX to XXXXX:

```
   1: ------------------------------------------------------------1
  61: 11111111111131333333333333333333333333333333333333333333333
```

```
121: 333333333333333333333333333333333333333333333333333333333333
181: 333333333333333333333333333333333333333333333333333333333333
241: 333333333333333333333333333333333333333333333333333333333333
301: 333333333333333333333333333333333333333333333333333333333333
361: 333333333333333333333333333333333333333333333333333333333333
421: 333333333333---------333333333333-------------------------
481: ----------------------------------------------------11---1111-
541: -111111111111111111111111111111111111111111111111111111111111
601: 111111111111111111111111111111111111111111111111111111111111
661: 111111111111111111111111111111111111111111111111111111111111
721: 111111111111111111111111111111111111111111111111111111111111
781: 111111111111111111111111111111111111111111111111111111111111
841: 111111111111111111111111111111111111111111111111111111111111
901: 111111111111111111111111111111111111111111111111111111111111
961: 111111111111111111111111111111111111111111111111111111111111
1021: 111111111111111111111111111111111111111111111111111111111111
1081: 1111111111111111111111111111111111111111----------------------
1141: ------------------------------------------------------------
1201: ------------------22222222222222221333333333333333333333333
1261: 333333333333333333333333333333333333333333333----------------
1321: ---333-----------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------3333-------33333-----------------------------3
2041: 33333333333333----13-22222222222222222222222222------------
2101: --22--------2222-12222222222222222222222222222222222222222
2161: 22222
```

XXYYXX to XXXXXX:

```
1: ------------------------------------------------------------1
61: 11111111111131333333333333333333333333333333333333333333333
121: 333333333333333333333333333333333333333333333333333333333333
181: 333333333333333333333333333333333333333333333333333333333333
241: 333333333333333333333333333333333333333333333333333333333333
301: 333333333333333333333333333333333333333333333333333333333333
361: 333333333333333333333333333333333333333333333333333333333333
421: 333333333333---------333333333333-------------------------
481: ----------------------------------------------------11111
541: 111111111111111111111111111111111111111111111111111111111111
601: 111111111111111111111111111111111111111111111111111111111111
661: 111111111111111111111111111111111111111111111111111111111111
721: 111111111111111111111111111111111111111111111111111111111111
781: 111111111111111111111111111111111111111111111111111111111111
841: 111111111111111111111111111111111111111111111111111111111111
```


901: 1111111111111111111111111111111111111111111111111111111111111111111
961: 1111111111111111111111111111111111111111111111111111111111111111111
1021: 1111111111111111111111111111111111111111111111111111111111111111111
1081: 11111111111111111111111111111111111111----------
1141: ----------
1201: ----------2222222222222221333333333333333333333333
1261: 333333333333333333333333333333333333333333333----------
1321: ---333----------
1381: ----------
1441: ----------
1501: ----------
1561: ----------
1621: ----------
1681: ----------
1741: ----------
1801: ----------
1861: ----------
1921: ----------
1981: ----------3333-------33333----------3
2041: 33333333333333----13-22222222222222222222222222----------
2101: ----------2222222222222222222222222222222222222222222222222
2161: 22222
XYYX to XXXX:
1: ----------1
61: 11111111111131333333333333333333333333333333333333333333333333333
121: 33333333333333333333333333333333333333333333333333333333333333333
181: 33333333333333333333333333333333333333333333333333333333333333333
241: 33333333333333333333333333333333333333333333333333333333333333333
301: 33333333333333333333333333333333333333333333333333333333333333333
361: 33333333333333333333333333333333333333333333333333333333333333333
421: 333333333333---------333333333333----------
481: ----------11111
541: 11111111111111111111111111111111111111111111111111111111111111111
601: 11111111111111111111111111111111111111111111111111111111111111111
661: 11111111111111111111111111111111111111111111111111111111111111111
721: 11111111111111111111111111111111111111111111111111111111111111111
781: 11111111111111111111111111111111111111111111111111111111111111111
841: 11111111111111111111111111111111111111111111111111111111111111111
901: 11111111111111111111111111111111111111111111111111111111111111111
961: 11111111111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111111111
1081: 11111111111111111111111111111111111111----------
1141: ----------
1201: ----------2222222222222221333333333333333333333333
1261: 333333333333333333333333333333333333333333333----------
1321: ---333----------
1381: ----------
1441: ----------
1501: ----------
1561: ----------
1621: ----------

```
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------3333-------33333----------------------------3
2041: 33333333333333----13-22222222222222222222222222-----------
2101: ------------222222222222222222222222222222222222222222222
2161: 22222
```

XYX to XXX:

```
   1: ------------------------------------------------------------1
  61: 11111111111113333333333333333333333333333333333333333333333
 121: 33333333333333333333333333333333333333333333333333333333333
 181: 33333333333333333333333333333333333333333333333333333333333
 241: 33333333333333333333333333333333333333333333333333333333333
 301: 33333333333333333333333333333333333333333333333333333333333
 361: 33333333333333333333333333333333333333333333333333333333333
 421: 333333333333---------333333333333---------------------------
 481: ------------------------------------------------------------11111
 541: 11111111111111111111111111111111111111111111111111111111111
 601: 11111111111111111111111111111111111111111111111111111111111
 661: 11111111111111111111111111111111111111111111111111111111111
 721: 11111111111111111111111111111111111111111111111111111111111
 781: 11111111111111111111111111111111111111111111111111111111111
 841: 11111111111111111111111111111111111111111111111111111111111
 901: 11111111111111111111111111111111111111111111111111111111111
 961: 11111111111111111111111111111111111111111111111111111111111
1021: 11111111111111111111111111111111111111111111111111111111111
1081: 1111111111111111111111111111111111111111--------------------
1141: ------------------------------------------------------------
1201: ------------------22222222222222213333333333333333333333333
1261: 333333333333333333333333333333333333333333333------------
1321: ---333-------------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------3333-------33333----------------------------3
2041: 33333333333333----13-22222222222222222222222222-----------
2101: ------------222222222222222222222222222222222222222222222
2161: 22222
```

Gaps between coding regions that are not introns are filled as before:

```
   1: ------------------------------------------------------------1
```

```
  61: 1111111111111133333333333333333333333333333333333333333333
 121: 3333333333333333333333333333333333333333333333333333333333
 181: 3333333333333333333333333333333333333333333333333333333333
 241: 3333333333333333333333333333333333333333333333333333333333
 301: 3333333333333333333333333333333333333333333333333333333333
 361: 3333333333333333333333333333333333333333333333333333333333
 421: 333333333333333333333333333333----------------------------
 481: ------------------------------------------------------11111
 541: 1111111111111111111111111111111111111111111111111111111111
 601: 1111111111111111111111111111111111111111111111111111111111
 661: 1111111111111111111111111111111111111111111111111111111111
 721: 1111111111111111111111111111111111111111111111111111111111
 781: 1111111111111111111111111111111111111111111111111111111111
 841: 1111111111111111111111111111111111111111111111111111111111
 901: 1111111111111111111111111111111111111111111111111111111111
 961: 1111111111111111111111111111111111111111111111111111111111
1021: 1111111111111111111111111111111111111111111111111111111111
1081: 11111111111111111111111111111111111111----------------------
1141: ------------------------------------------------------------
1201: ------------------22222222222222213333333333333333333333333
1261: 3333333333333333333333333333333333333333333333333333333333
1321: 333333------------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------333333333333333333333333333333333333333333333
2041: 3333333333333331113222222222222222222222222222222222222222222
2101: 2222222222222222222222222222222222222222222222222222222222222
2161: 22222
```

Frameshifts are verified and nucleotides are reclassified accordingly:

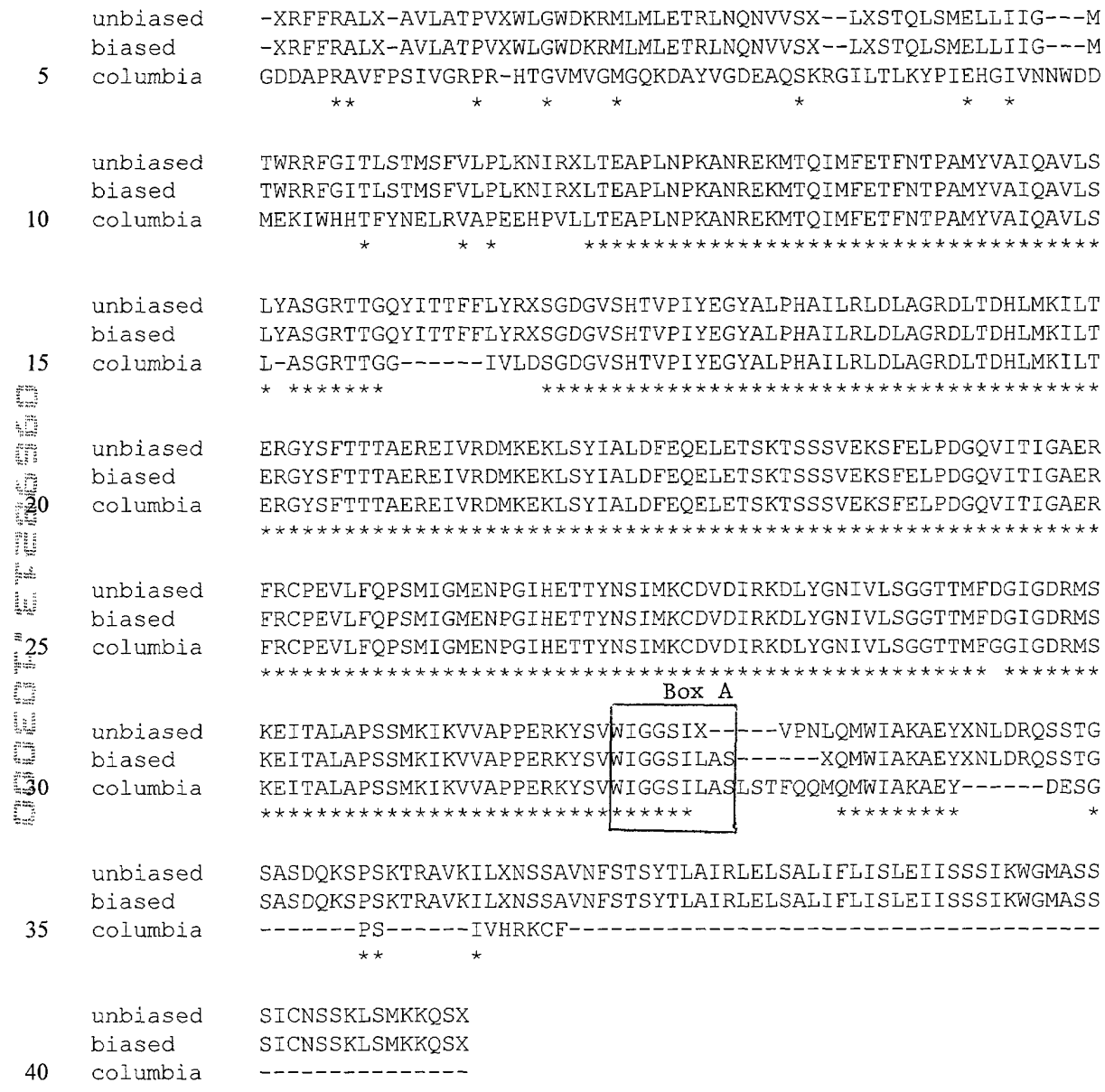
```
   1: ------------------------------------------------------------1
  61: 1111111111111111111111111111111111111111111111111111111111111
 121: 1111111111111111111111111111111111111111111111111111111111111
 181: 1111111111111111111111111111111111111111111111111111111111111
 241: 1111111111111111111111111111111111111111111333333333333333333
 301: 3333333333333333333333333333333333333333333333333333333333333
 361: 3333333333333333333333333333333333333333333333333333333333333
 421: 333333333333333333333333333333----------------------------
 481: ------------------------------------------------------11111
 541: 1111111111111111111111111111111111111111111111111111111111111
 601: 1111111111111111111111111111111111111111111111111111111111111
 661: 1111111111111111111111111111111111111111111111111111111111111
 721: 1111111111111111111111111111111111111111111111111111111111111
 781: 1111111111111111111111111111111111111111111111111111111111111
```

```
 841: 1111111111111111111111111111111111111111111111111111111111111111
 901: 1111111111111111111111111111111111111111111111111111111111111111
 961: 1111111111111111111111111111111111111111111111111111111111111111
1021: 1111111111111111111111111111111111111111111111111111111111111111
1081: 11111111111111111111111111111111111----------------------------------
1141: ------------------------------------------------------------
1201: ------------------222222222222222222222222233333333333333
1261: 333333333333333333333333333333333333333333333333333333333333
1321: 333333------------------------------------------------------------
1381: ------------------------------------------------------------
1441: ------------------------------------------------------------
1501: ------------------------------------------------------------
1561: ------------------------------------------------------------
1621: ------------------------------------------------------------
1681: ------------------------------------------------------------
1741: ------------------------------------------------------------
1801: ------------------------------------------------------------
1861: ------------------------------------------------------------
1921: ------------------------------------------------------------
1981: --------------333333333333333333333333333333333333333333333
2041: 333333333333333333333333333333332222222222222222222222222222
2101: 222222222222222222222222222222222222222222222222222222222222
2161: 22222
```

And the sequence is translated as before:

```
  1 : XRFFRALxAVLATPVxWLGWDKRMLMLETRLNQNVVSxLxSTQLSMELLIIGMTWRRFGI
 61 : TLSTMSFVLPLKNIRXLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRT
121 : TGQYITTFFLYRXSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDHLMKILTERGYSFT
181 : TTAEREIVRDMKEKLSYIALDFEQELETSKTSSSVEKSFELPDGQVITIGAERFRCPEVL
241 : FQPSMIGMENPGIHETTYNSIMKCDVDIRKDLYGNIVLSGGTTMFDGIGDRMSKEITALA
301 : PSSMKIKVVAPPERKYSVWIGGSILASXQMWIAKAEYXNLDRQSSTGSASDQKSPSKTRA
361 : VKILXNSSAVNFSTSYTLAIRLELSALIFLISLEIISSSIKWGMASSSICNSSKLSMKKQ
421 : SX (SEQ. ID. NO.3)
```

The resulting amino acid sequence (SEQ. ID. NO. 3) differs from the amino acid sequence calculated without a bias (SEQ. ID. NO. 2). The relative accuracy of the two amino acid sequences can be determined by comparison to a known sequence. SEQ. ID. NO. 2 and SEQ. ID. NO. 3 are compared to the translation of the actin gene from *Arabidopsis thaliana, columbia* (SEQ. ID. NO. 4). Dashes indicate gaps in the sequence and asterisks indicate a match among all three sequences. The predicted amino acid sequences (SEQ. ID. NOs. 2 and 3) are based on an *Arabidopsis thaliana, landsberg* ecotype. A comparison of the predicted with a known *Arabidopsis thaliana, columbia* ecotype amino acid sequence (SEQ. ID. NO. 4) is shown below. The sequence set forth in Box A illustrates an area of the biased sequence that shows a higher level of identity with the *Arabidopsis thaliana, columbia* sequence.


unbiased -XRFFRALX-AVLATPVXWLGWDKRMLMLETRLNQNVVSX--LXSTQLSMELLIIG---M
biased -XRFFRALX-AVLATPVXWLGWDKRMLMLETRLNQNVVSX--LXSTQLSMELLIIG---M
columbia GDDAPRAVFPSIVGRPR-HTGVMVGMGQKDAYVGDEAQSKRGILTLKYPIEHGIVNNWDD
unbiased TWRRFGITLSTMSFVLPLKNIRXLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLS
biased TWRRFGITLSTMSFVLPLKNIRXLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLS
columbia MEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREKMTQIMFETFNTPAMYVAIQAVLS
unbiased LYASGRTTGQYITTFFLYRXSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDHLMKILT
biased LYASGRTTGQYITTFFLYRXSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDHLMKILT
columbia L-ASGRTTGG------IVLDSGDGVSHTVPIYEGYALPHAILRLDLAGRDLTDHLMKILT
unbiased ERGYSFTTTAEREIVRDMKEKLSYIALDFEQELETSKTSSSVEKSFELPDGQVITIGAER
biased ERGYSFTTTAEREIVRDMKEKLSYIALDFEQELETSKTSSSVEKSFELPDGQVITIGAER
columbia ERGYSFTTTAEREIVRDMKEKLSYIALDFEQELETSKTSSSVEKSFELPDGQVITIGAER
unbiased FRCPEVLFQPSMIGMENPGIHETTYNSIMKCDVDIRKDLYGNIVLSGGTTMFDGIGDRMS
biased FRCPEVLFQPSMIGMENPGIHETTYNSIMKCDVDIRKDLYGNIVLSGGTTMFDGIGDRMS
columbia FRCPEVLFQPSMIGMENPGIHETTYNSIMKCDVDIRKDLYGNIVLSGGTTMFGGIGDRMS
Box A
unbiased KEITALAPSSMKIKVVAPPERKYSVWIGGSIX------VPNLQMWIAKAEYXNLDRQSSTG
biased KEITALAPSSMKIKVVAPPERKYSVWIGGSILAS------XQMWIAKAEYXNLDRQSSTG
columbia KEITALAPSSMKIKVVAPPERKYSVWIGGSILASLSTFQQMQMWIAKAEY------DESG
unbiased SASDQKSPSKTRAVKILXNSSAVNFSTSYTLAIRLELSALIFLISLEIISSSIKWGMASS
biased SASDQKSPSKTRAVKILXNSSAVNFSTSYTLAIRLELSALIFLISLEIISSSIKWGMASS
columbia -------PS------IVHRKCF-----------------------------------
unbiased SICNSSKLSMKKQSX
biased SICNSSKLSMKKQSX
columbia ---------------

We Claim

1. A method for determining a probability for one or more states for a nucleotide in a nucleic acid sequence, comprising:

a) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in said nucleic acid sequence;

b) determining transition probabilities for each of said states for nucleotides within said nucleic acid sequence following said initial oligonucleotide;

c) determining a probability for said nucleic acid sequence for each of said states; and,

d) determining a probability for each of said states for said nucleotide based upon said probability of said nucleic acid sequence and a bias.

2. The method of claim 1, wherein said probability for each of said states for said nucleotide is determined using an inhomogeneous Markov model having eight states, wherein said eight states are: first reading frame positive strand (1+); second reading frame positive strand (2+); third reading frame positive strand (3+); first reading frame negative strand (1-); second reading frame negative strand (2-); third reading frame negative strand (3-); noncoding positive strand (N+); and, noncoding negative strand (N-).

3. The method of claim 2, wherein said probability for each of said eight states for said nucleotide in step e) is determined using the equation

$$P(f|S) = \frac{\phi(f) \cdot P_f \cdot P_f(S)}{\sum_{i \in \{1+,2+,3+,N+,1-,2-,3-,N-\}} \phi(f) \cdot P_i \cdot P_i(S)}$$

4. The method of claim 1, wherein said nucleotide is the middle nucleotide in said nucleic acid sequence.

5. The method of claim 1, wherein said nucleic acid sequence is part of a longer nucleic acid sequence.

6. The method of claim 1, wherein said bias is between 0.0 and 0.9 or greater than 1.1.

7. A method for determining a probability for one or more states for a nucleotide in a nucleic acid sequence, comprising:

a) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in said nucleic acid sequence;

b) determining transition probabilities for each of said states for nucleotides within said nucleic acid sequence following said initial oligonucleotide;

c) determining a probability for said nucleic acid sequence for each of said states; and,

d) determining a probability for each of said states for said nucleotide based upon said probability of said nucleic acid sequence, wherein said determining a probability for each of said states is capable of accepting a bias.

8. A method for determining a probability for each of one or more states for more than one nucleotide in a nucleic acid sequence comprising:

a) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in a window of a first nucleotide;

b) determining transition probabilities for each of said states for nucleotides within said window following said initial oligonucleotide;

c) determining a probability for said window for each of said states;

d) determining a probability for each of said states for said nucleotide based upon said probability for said window and a bias; and,

e) repeating steps a) through d) for each remaining nucleotide in said nucleic acid sequence.

9. The method of claim 8, wherein said more than one nucleotide are contiguous, and step e) is performed sequentially from said first nucleotide to a last nucleotide.

10. The method of claim 9, wherein said probability for each of said states for said more than one nucleotide is determined using an inhomogeneous Markov model having eight states, wherein said eight states are: first reading frame positive strand (1+); second reading frame positive strand (2+); third reading frame positive strand (3+); first reading frame negative strand (1-); second reading frame negative strand (2-); third reading frame negative strand (3-); noncoding positive strand (N+); and, noncoding negative strand (N-).

11. The method of claim 10, wherein said probability for each of said states for said more than one nucleotide is determined using the equation

$$P(f|S) = \frac{\phi(f) \cdot P_f \cdot P_f(S)}{\sum_{i \in \{1+,2+,3+,N+,1-,2-,3-,N-\}} \phi(f) \cdot P_i \cdot P_i(S)}$$

12. The method of claim 8, wherein said nucleic acid sequence is part of a longer nucleic acid sequence.

13. The method of claim 8, wherein each nucleotide in said more than one nucleotide is the middle nucleotide in its own window.

14. The method of claim 8, further comprising:

f) extending said nucleic acid sequence if said window extends beyond either end of said nucleic acid sequence, wherein said extending is accomplished by copying nucleotides from an end of said nucleic acid sequence at which said window is located to produce a copied nucleotide sequence, and adding said copied nucleotide sequence to said end.

15. The method of claim 8, wherein said window has a length of about 75 to about 125.

16. The method of claim 8, wherein said bias is between 0.0 and 0.9 or greater than 1.1.

17. A method for determining strand coding of a nucleic acid sequence, comprising:

a) determining a probability of each of one or more states for each nucleotide in said nucleic acid sequence based upon a bias, wherein each of said states is either a positive strand state or a negative strand state;

b) summing said probabilities of said positive strand states for each of said nucleotides to produce a sum of probabilities for positive states;

c) summing said probabilities of said negative strand states for each of said nucleotides to produce a sum of probabilities for negative states; and,

d) deciding one of

i) coding is mixed or not detectable if a first function of said sum of probabilities for positive states and said sum of probabilities for negative states is less than a threshold value;

ii) coding is on said positive strand if a second function of said sum of probabilities for positive states is greater than a third function of said sum of probabilities for negative states and said first function is not less than said threshold value; and

iii) coding is on said negative strand if said second function of said sum of probabilities for positive states is not greater than said third function of said sum of probabilities for negative states and said first function is not less than said threshold value.

18. The method of claim 17, wherein said sum of probabilities for positive states is X, said sum of probabilities for negative states is Y and said first function is $f(X,Y) = \frac{|X-Y|}{(X+Y)}$.

19. The method of claim 18, wherein said threshold value is from about 0.4 to about 0.6.

20. The method of Claim 17, wherein said sum of probabilities for positive states is X, said sum of probabilities for negative states is Y, said second function is f(X)=X, and said third function is f(Y)=Y.

21. The method of claim 17, wherein step a) comprises:

e) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in a window of a first nucleotide;

f) determining transition probabilities for each of said states for nucleotides within said window following said initial oligonucleotide;

g) determining a probability for said window for each of said states;

h) determining a probability for each of said states for said nucleotide based upon said probability for said window and a bias; and,

i) repeating steps e) through h) for each remaining nucleotide in said nucleic acid sequence.

22. A method for determining the extent of an open reading frame within a nucleic acid sequence, comprising:

a) determining the probability of each of one or more states for each nucleotide in said nucleic acid sequence based upon a bias, wherein each of said states is either a coding state or a noncoding state;

b) determining the coding strand of said nucleic acid sequence; and,

c) determining the points within said nucleic acid sequence in said coding strand at which the sum of the probabilities of said coding states for each nucleotide drops below a first threshold value for a number of nucleotides greater than a second threshold value, wherein ends of said open reading frame are indicated at said points.

23. The method of claim 22, wherein said first threshold value is about 0.4 to about 0.6.

24. The method of claim 22, wherein said second threshold value is about 500 to about 700.

25. The method of claim 22, wherein step c) comprises:

d) determining the sum of said coding states for a middle nucleotide located in said nucleic acid sequence;

e) repeating step d) sequentially for nucleotides located on a first side of said middle nucleotide until either

i) the sum of the probabilities of said coding states drops below said first threshold value for a number of nucleotides greater than said second threshold value, or

ii) an end of said nucleic acid sequence is reached,

at which point an end of the open reading frame is indicated; and,

f) repeating step e) for nucleotides located on a second side of said middle nucleotide.

26. The method of claim 22, wherein said nucleic acid sequence is part of a longer nucleic acid sequence.

27. The method of claim 22, wherein step b) comprises

d) summing probabilities of positive strand states for each of said nucleotides to produce a sum of probabilities for positive states;

e) summing probabilities of negative strand states for each of said nucleotides to produce a sum of probabilities for negative states; and,

f) deciding one of

i) coding is mixed or not detectable if a first function of said sum of probabilities for positive states and said sum of probabilities for negative states is less than a threshold value;

ii) coding is on said positive strand if a second function of said sum of probabilities for positive states is greater than a third function of said sum of probabilities for negative states and said first function is not less than said threshold value; and

iii) coding is on said negative strand if said second function of said sum of probabilities for positive states is not greater than said third function of said sum of probabilities for negative states and said first function is not less than said threshold value.

28. The method of claim 22, wherein step a) comprises:

d) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in a window of a first nucleotide;

e) determining transition probabilities for each of said states for nucleotides within said window following said initial oligonucleotide;

f) determining a probability for said window for each of said states;

g) determining a probability for each of said states for said nucleotide based upon said probability for said window and a bias; and,

h) repeating steps d) through g) for each remaining nucleotide in said nucleic acid sequence.

29. A method for determining the location of insertions and deletions within a nucleic acid sequence, comprising:

a) determining the probability of each of one or more states for each nucleotide in said nucleic acid sequence based upon a bias, wherein each of said states is either a coding state or a noncoding state;

b) setting a length for a window;

c) determining which state has a maximum mean probability for said nucleic acid sequence on a first side of a middle nucleotide in said window, wherein said window begins at a first nucleotide;

d) determining which state has a maximum mean probability for said nucleic acid sequence on a second side of said middle nucleotide in said window;

e) determining that a deletion or insertion occurred at said middle nucleotide if

i) said state with said maximum mean probability on said first side of said middle nucleotide is different from said state with said maximum mean probability on said second side of middle nucleotide, and

ii) either an average of hypothetical state probabilities for said window with an insertion at said middle nucleotide or an average of hypothetical state probabilities for said window with a deletion at said middle nucleotide is greater than a sum of said middle nucleotide's coding states probabilities; and,

f) repeating steps c) through e) for each remaining nucleotide in said nucleic acid sequence after said first nucleotide, wherein said window begins at each remaining nucleotide in turn.

30. The method of claim 29, further comprising:

g) determining that a deletion occurred if said average of hypothetical state probabilities for said window with an insertion at said middle nucleotide is greater than an average hypothetical state probabilities for said window with a deletion at said middle nucleotide or that an insertion occurred if said average hypothetical state probabilities for said window with an insertion at said middle nucleotide is not greater than an average of hypothetical state probabilities for said window with a deletion at said middle nucleotide.

31. The method of claim 29, wherein said nucleic acid sequence is part of a longer nucleic acid sequence.

32. The method of claim 29, wherein said repeating in step f) is performed sequentially from said first nucleotide to a last nucleotide.

33. The method of claim 29, wherein said window is about 75 to about 125.

34. The method of claim 29, wherein step a) comprises:

g) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in a window of a first nucleotide;

h) determining transition probabilities for each of said states for nucleotides within said window following said initial oligonucleotide;

i) determining a probability for said window for each of said states;

j) determining a probability for each of said states for said nucleotide based upon said probability for said window and a bias; and,

k) repeating steps g) through j) for each remaining nucleotide in said nucleic acid sequence.

35. A method for determining exon location within a nucleic acid sequence, comprising

a) determining the probability of each of one or more states for each nucleotide in said nucleic acid sequence based upon a bias, wherein each of said states is either a coding state or noncoding state;

b) determining the coding strand of said nucleic acid sequence;

c) determining the extent of an open reading frame within said nucleic acid sequence;

d) classifying each nucleotide in a coding class or a noncoding class based on a most probable state for said coding strand;

e) reclassifying each nucleotide according to defined rules; and,

f) determining that regions of said nucleic acid sequence in said coding class are exons.

36. The method of claim 35, wherein step e) comprises:

g) reclassifying a noncoding nucleotide to a class of an adjacent nucleotide on a first side of said noncoding nucleotide and an adjacent nucleotide on a second side of said noncoding nucleotide if said adjacent nucleotide on said first side and said adjacent nucleotide on said second side all are of a single class;

h) reclassifying a nucleotide to a class of two adjacent nucleotides on a first side and two adjacent nucleotides on a second side if said two adjacent nucleotides on said first side and said two adjacent nucleotides on said second side all are of a single class;

i) reclassifying a first pair of adjacent nucleotides having a same class to a class of two adjacent nucleotides on a first side of said first pair and two adjacent nucleotides on a second side of said first pair if said two adjacent nucleotides on said first side and said two adjacent nucleotides on said second side all are of a single class;

j) reclassifying a second pair of adjacent nucleotides having a same class to a class of an adjacent nucleotide on a first side of said second pair and an adjacent nucleotide on a second side of said second pair if said adjacent nucleotide on said first side and said adjacent nucleotide on said second side both are of a single class;

k) reclassifying a nucleotide to a class of an adjacent nucleotide on a first side of said single nucleotide and an adjacent nucleotide on a second side of said nucleotide if said adjacent

nucleotide on said first side and said adjacent nucleotide on said second side both are of a single class;

l) reclassifying a continuous sequence of less than a defined minimum number of nucleotides in a noncoding class having nucleotides in a coding class on both sides to a coding class of flanking nucleotides; and,

m) reclassifying a coding segment comprising more than one class of nucleotides to a most common class in said segment.

37. The method of claim 35, wherein step b) comprises:

g) summing probabilities of positive strand states for each of said nucleotides to produce a sum of probabilities for positive states;

h) summing probabilities of negative strand states for each of said nucleotides to produce a sum of probabilities for negative states; and,

i) deciding one of

I) coding is mixed or not detectable if a first function of said sum of probabilities for positive states and said sum of probabilities for negative states is less than a threshold value;

II) coding is on said positive strand if a second function of said sum of probabilities for positive states is greater than a third function of said sum of probabilities for negative states and said first function is not less than said threshold value; and

III) coding is on said negative strand if said second function of said sum of probabilities for positive states is not greater than said third function of said sum of probabilities for negative states and said first function is not less than said threshold value.

38. The method of claim 35, wherein step c) comprises:

g) determining the points within said nucleic acid sequence in said coding strand at which a sum of the probabilities of coding states for each nucleotide drops below a first threshold value for a number of nucleotides greater than a second threshold value, wherein ends of an open reading frame are indicated at said points.

39. The method of claim 35, wherein step a) comprises:

g) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in a window of a first nucleotide;

h) determining transition probabilities for each of said states for nucleotides within said window following said initial oligonucleotide;

i) determining a probability for said window for each of said states;

j) determining a probability for each of said states for said nucleotide based upon said probability for said window and a bias; and,

k) repeating steps g) through j) for each remaining nucleotide in said nucleic acid sequence.

40. The method of claim 35, further comprising:

g) translating said exons to determine a protein sequence.

41. A method for determining a probability for one or more states for a nucleotide in a nucleic acid sequence, comprising determining a probability for each of said states for said nucleotide based upon a probability of said nucleic acid sequence and a bias.

42. A method for determining a probability for each of one or more states for more than one nucleotide in a nucleic acid sequence comprising:

a) determining a probability for each of said states for a first nucleotide in said nucleic acid sequence based upon a probability of a window in which said first nucleotide is located and a bias; and,

b) repeating step a) for the remaining nucleotides in said nucleic acid sequence.

43. A program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine a probability for each of one or more states for a nucleotide in a nucleic acid sequence, said method steps comprising:

a) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in said nucleic acid sequence;

b) determining transition probabilities for each of said states for nucleotides within said nucleic acid sequence following said initial oligonucleotide;

c) determining a probability for said nucleic acid sequence for each of said states; and,

d) determining a probability for each of said states for said nucleotide based upon said probability of said nucleic acid sequence and a bias.

44. A program storage device readable by a machine, tangibly embodying a program of instructions executable by a machine to perform method steps to determine a probability for one or more states for more than one nucleotide in a nucleic acid sequence, said method steps comprising:

a) determining an initial oligonucleotide probability for each of said states for an initial oligonucleotide in a window of a first nucleotide;

b) determining transition probabilities for each of said states for nucleotides within said window following said initial oligonucleotide;

c) determining a probability for said window for each of said states;

d) determining a probability for each of said states for said nucleotide based upon said probability for said window and a bias; and,

e) repeating steps a) through d) for each remaining nucleotide in said nucleic acid sequence.

Abstract

The present invention is in the field of bioinformatics, particularly as it pertains to gene prediction. More specifically, the invention relates to the probabilistic analysis of nucleic acid sequences for the determination of coding features, including determination of state probabilities for each nucleotide in a nucleic acid sequence, determination of coding strand, determination of open reading frame extent, determination of insertion and deletion location, determination of exon location, and determination of protein sequence.

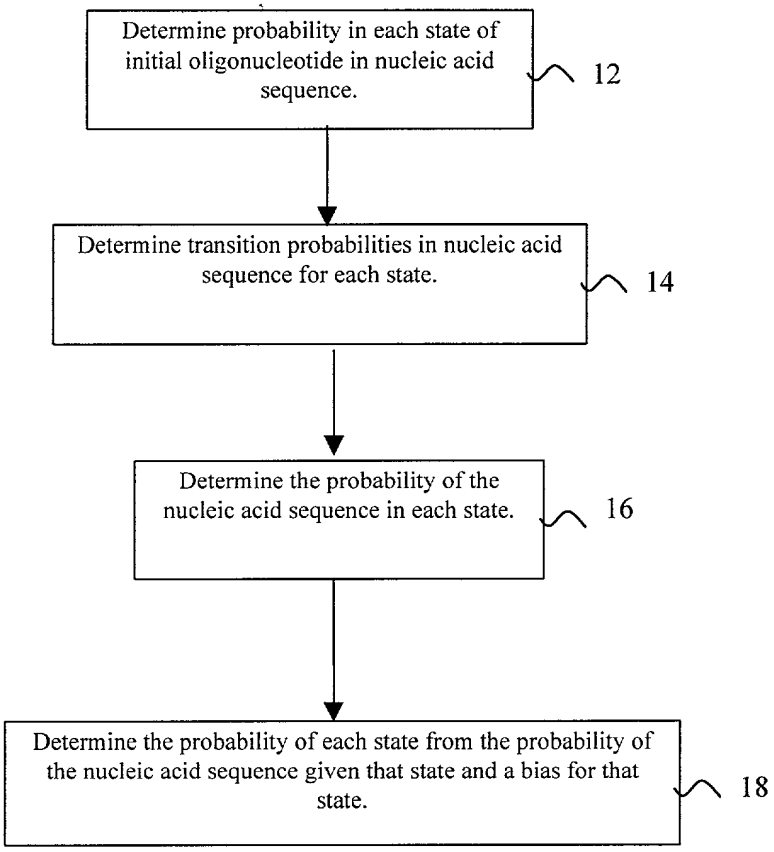

Determine probability in each state of initial oligonucleotide in nucleic acid sequence.
12
Determine transition probabilities in nucleic acid sequence for each state.
14
Determine the probability of the nucleic acid sequence in each state.
16
Determine the probability of each state from the probability of the nucleic acid sequence given that state and a bias for that state.
18

Figure 1

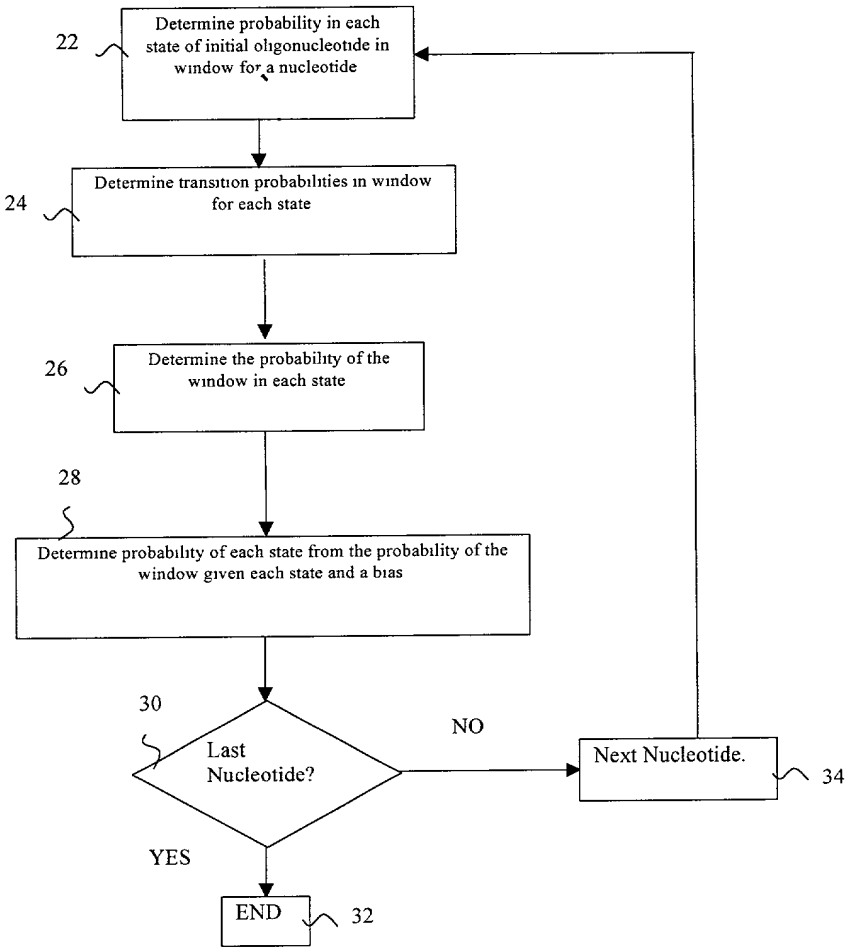

22
Determine probability in each state of initial oligonucleotide in window for a nucleotide
24
Determine transition probabilities in window for each state
26
Determine the probability of the window in each state
28
Determine probability of each state from the probability of the window given each state and a bias
30
Last Nucleotide?
NO
Next Nucleotide.
34
YES
END
32

Figure 2

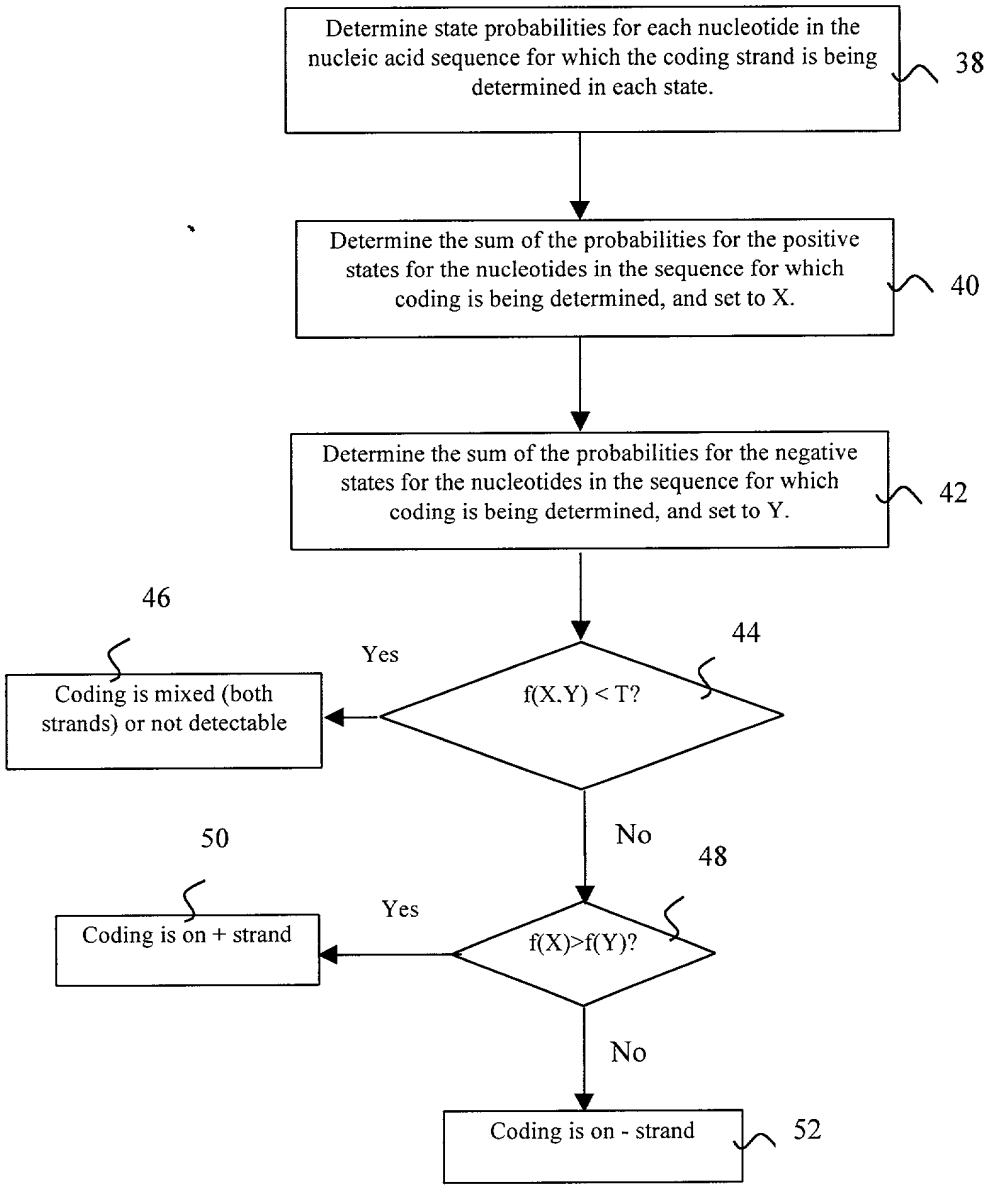

Determine state probabilities for each nucleotide in the nucleic acid sequence for which the coding strand is being determined in each state.
38
Determine the sum of the probabilities for the positive states for the nucleotides in the sequence for which coding is being determined, and set to X.
40
Determine the sum of the probabilities for the negative states for the nucleotides in the sequence for which coding is being determined, and set to Y.
42
46
Yes
44
Coding is mixed (both strands) or not detectable
f(X.Y) < T?
No
50
48
Yes
Coding is on + strand
f(X)>f(Y)?
No
Coding is on - strand
52

Figure 3

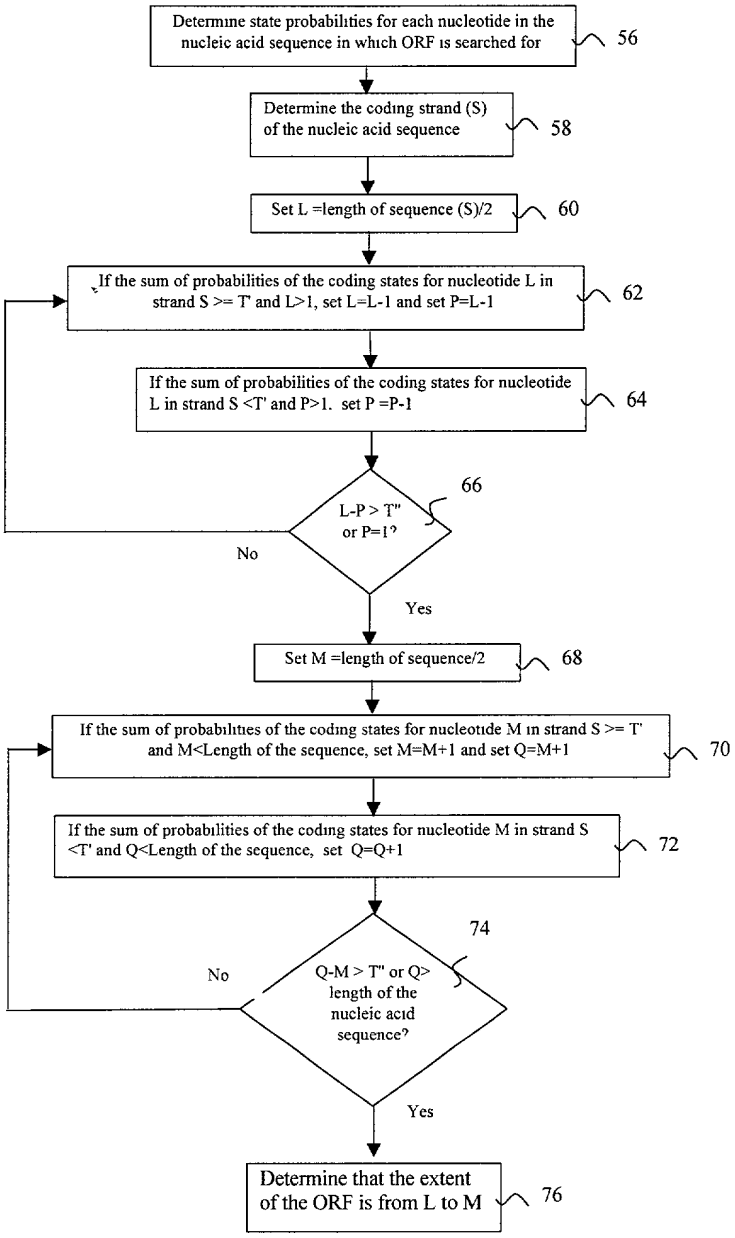

Determine state probabilities for each nucleotide in the nucleic acid sequence in which ORF is searched for
56
Determine the coding strand (S) of the nucleic acid sequence
58
Set L =length of sequence (S)/2
60
If the sum of probabilities of the coding states for nucleotide L in strand S >= T' and L>1, set L=L-1 and set P=L-1
62
If the sum of probabilities of the coding states for nucleotide L in strand S <T' and P>1. set P =P-1
64
L-P > T" or P=1?
66
No
Yes
Set M =length of sequence/2
68
If the sum of probabilities of the coding states for nucleotide M in strand S >= T' and M<Length of the sequence, set M=M+1 and set Q=M+1
70
If the sum of probabilities of the coding states for nucleotide M in strand S <T' and Q<Length of the sequence, set Q=Q+1
72
Q-M > T" or Q> length of the nucleic acid sequence?
74
No
Yes
Determine that the extent of the ORF is from L to M
76

Figure 4

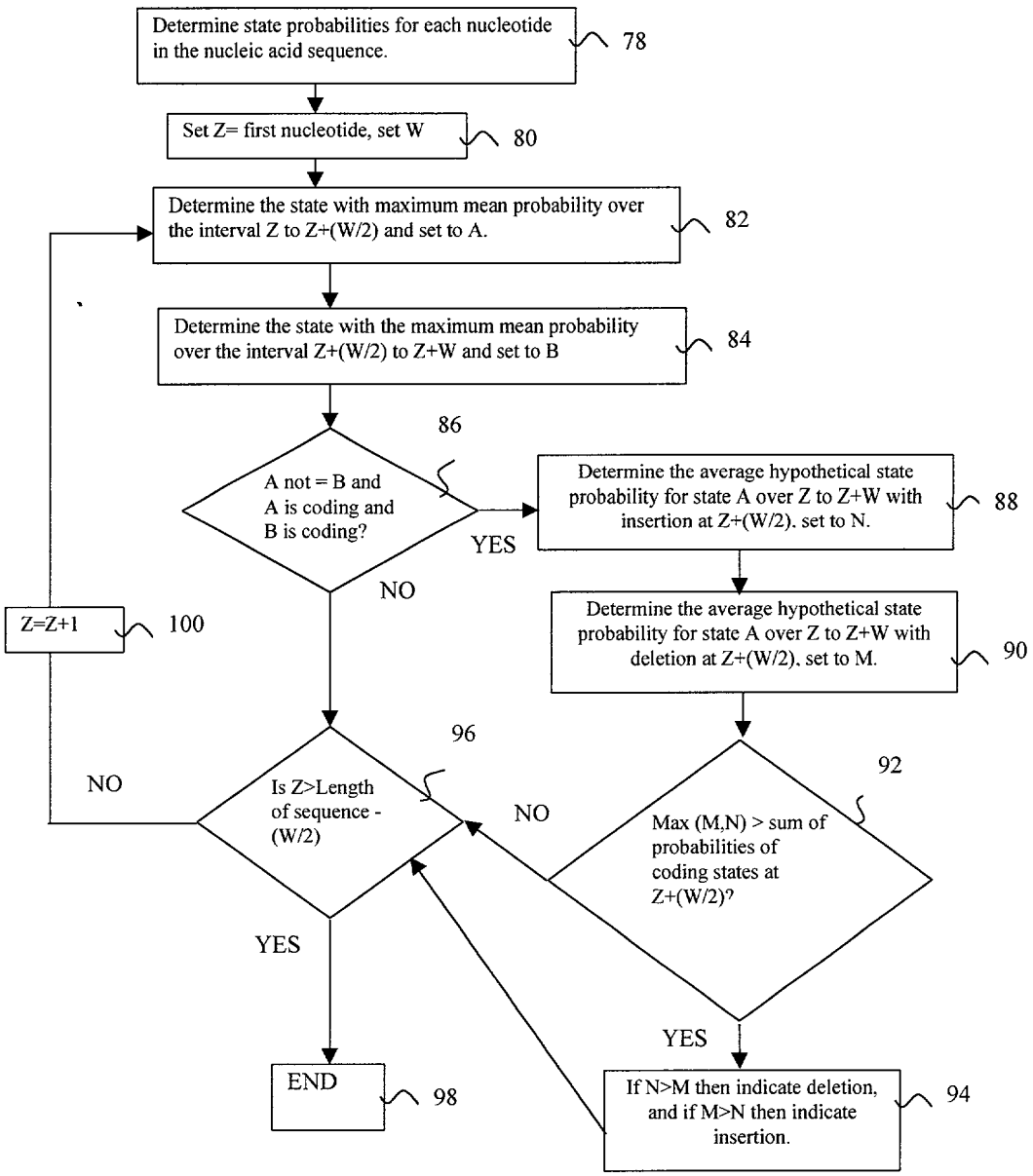

Determine state probabilities for each nucleotide in the nucleic acid sequence.
78
Set Z= first nucleotide, set W
80
Determine the state with maximum mean probability over the interval Z to Z+(W/2) and set to A.
82
Determine the state with the maximum mean probability over the interval Z+(W/2) to Z+W and set to B
84
86
A not = B and A is coding and B is coding?
YES
Determine the average hypothetical state probability for state A over Z to Z+W with insertion at Z+(W/2), set to N.
88
NO
Determine the average hypothetical state probability for state A over Z to Z+W with deletion at Z+(W/2), set to M.
90
Z=Z+1
100
96
Is Z>Length of sequence - (W/2)
NO
92
Max (M,N) > sum of probabilities of coding states at Z+(W/2)?
NO
YES
YES
END
98
If N>M then indicate deletion, and if M>N then indicate insertion.
94

Figure 5

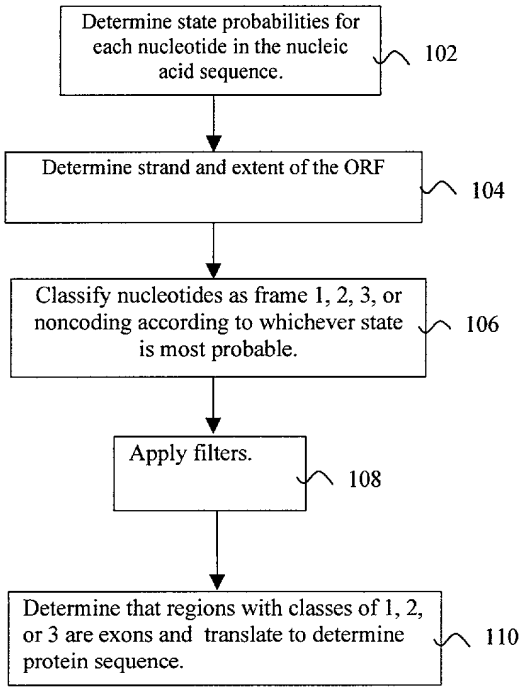

Determine state probabilities for each nucleotide in the nucleic acid sequence.
102
Determine strand and extent of the ORF
104
Classify nucleotides as frame 1, 2, 3, or noncoding according to whichever state is most probable.
106
Apply filters.
108
Determine that regions with classes of 1, 2, or 3 are exons and translate to determine protein sequence.
110

Figure 6

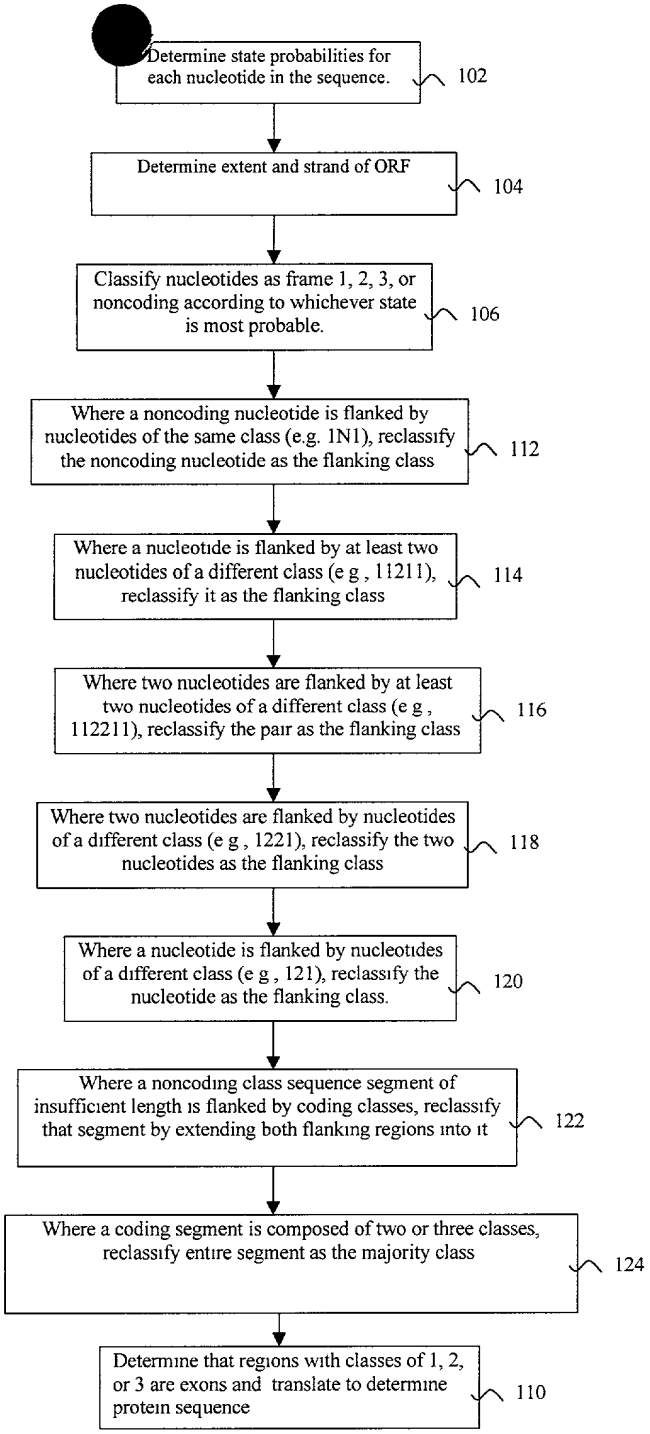

Determine state probabilities for each nucleotide in the sequence.
102
Determine extent and strand of ORF
104
Classify nucleotides as frame 1, 2, 3, or noncoding according to whichever state is most probable.
106
Where a noncoding nucleotide is flanked by nucleotides of the same class (e.g. 1N1), reclassify the noncoding nucleotide as the flanking class
112
Where a nucleotide is flanked by at least two nucleotides of a different class (e g , 11211), reclassify it as the flanking class
114
Where two nucleotides are flanked by at least two nucleotides of a different class (e g , 112211), reclassify the pair as the flanking class
116
Where two nucleotides are flanked by nucleotides of a different class (e g , 1221), reclassify the two nucleotides as the flanking class
118
Where a nucleotide is flanked by nucleotides of a different class (e g , 121), reclassify the nucleotide as the flanking class.
120
Where a noncoding class sequence segment of insufficient length is flanked by coding classes, reclassify that segment by extending both flanking regions into it
122
Where a coding segment is composed of two or three classes, reclassify entire segment as the majority class
124
Determine that regions with classes of 1, 2, or 3 are exons and translate to determine protein sequence
110

Figure 7

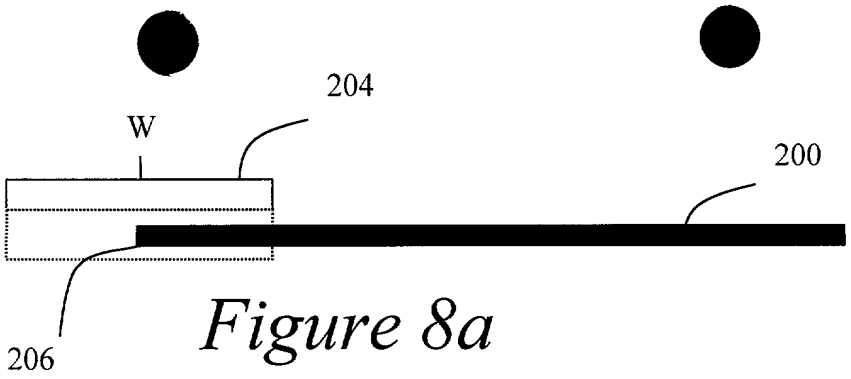


Figure 8a

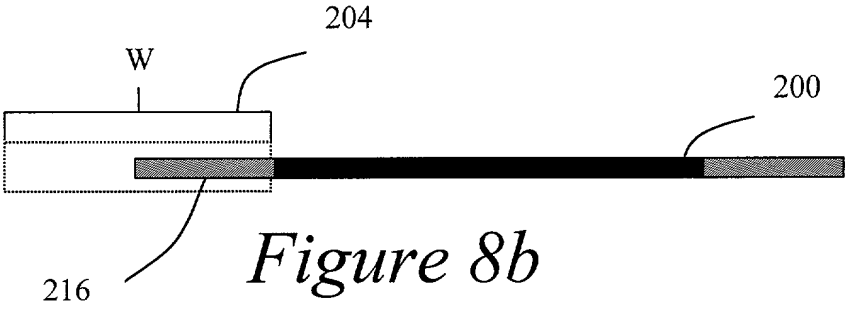


Figure 8b

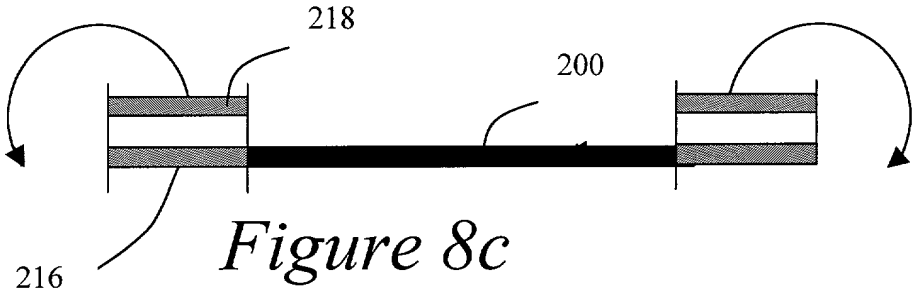
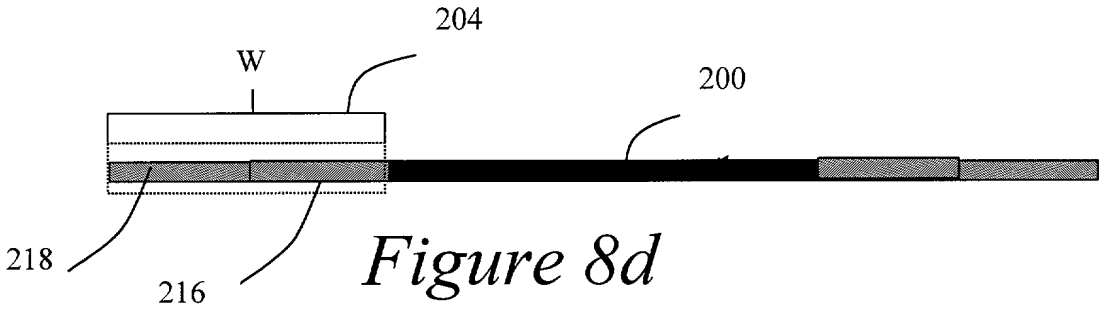


Figure 8c



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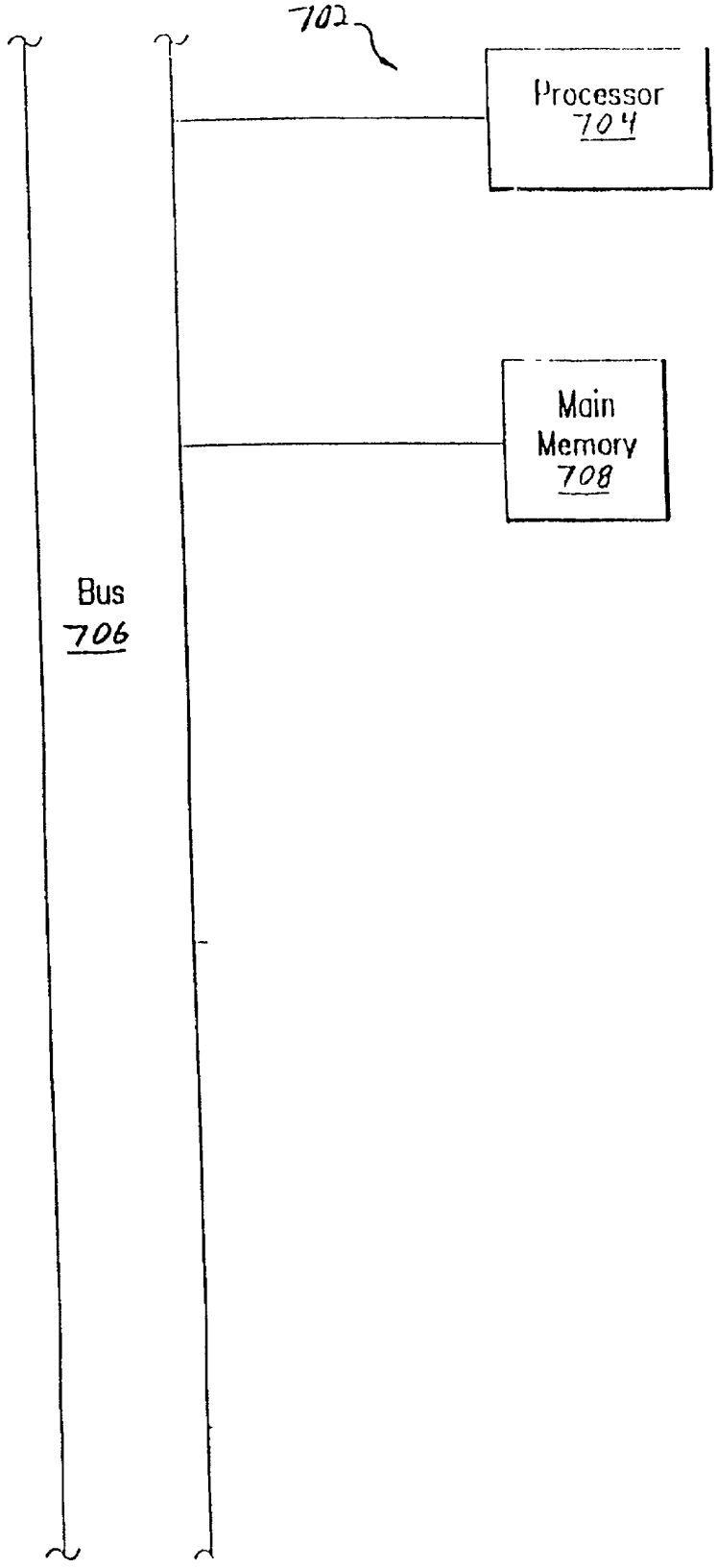
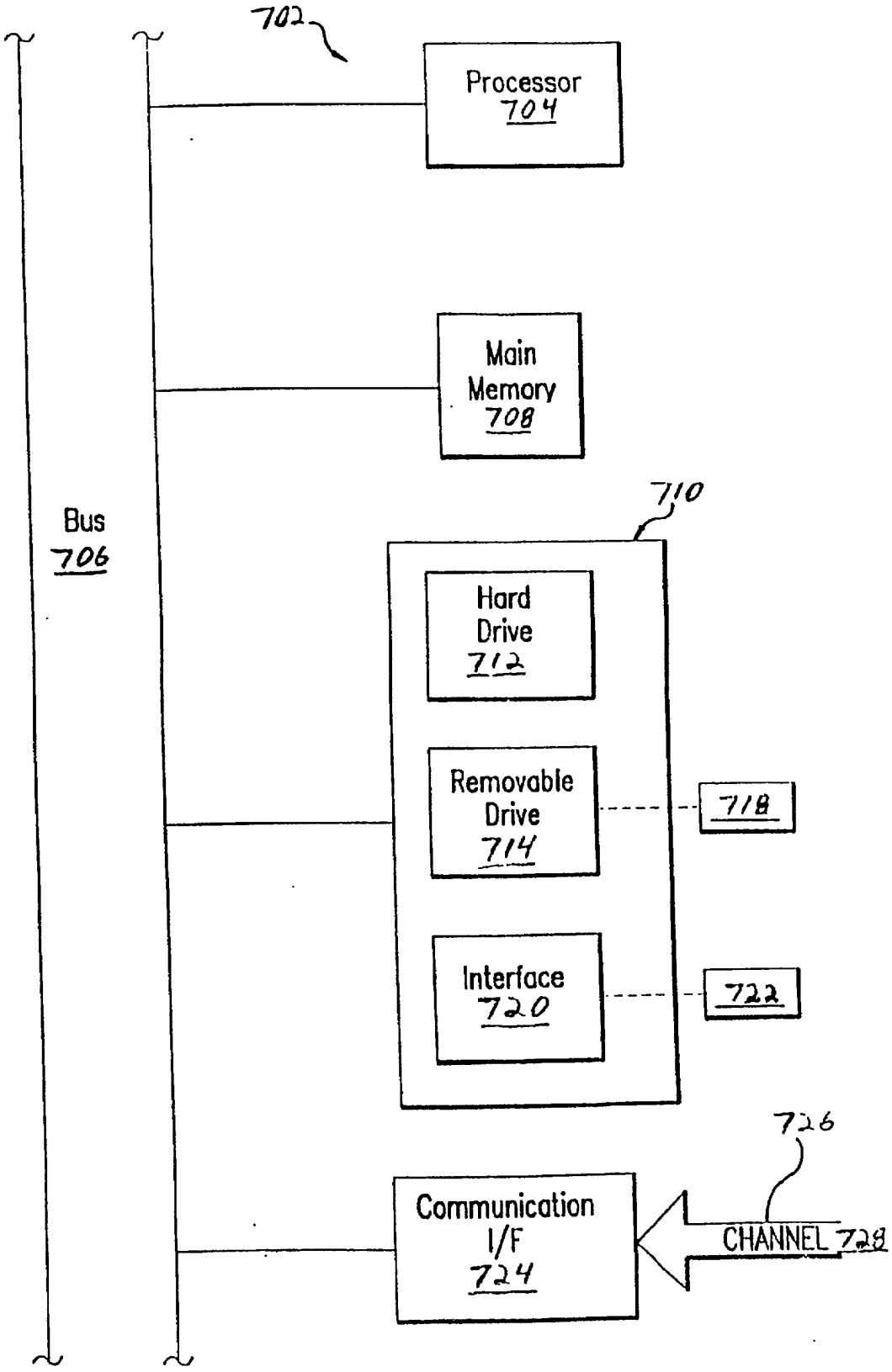


Figure 9a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I/F
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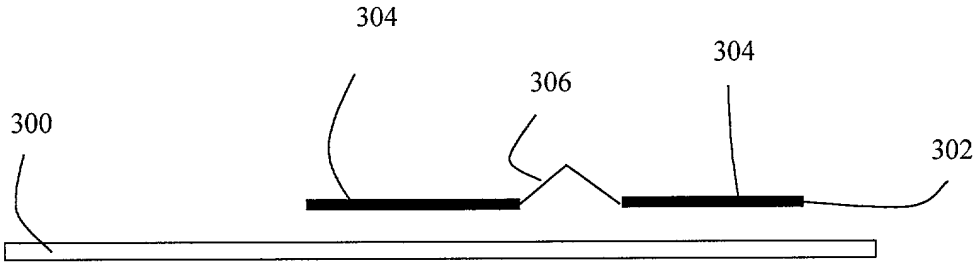
CHANNEL 728

Figure 9b



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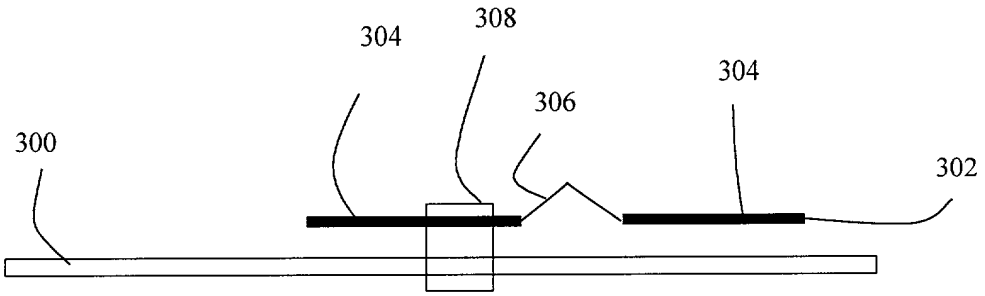

Figure 10b

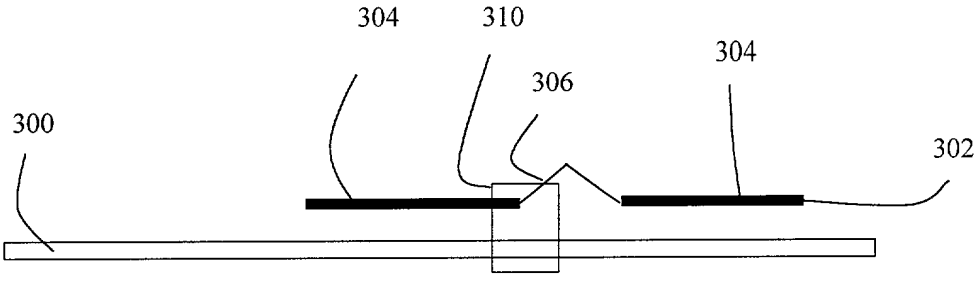

Figure 10c

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

(A) This declaration is for an original application.

(B) My residence, post office address and citizenship are as stated below, next to my name. I believe that I am an original, first and sole inventor of the subject matter that is claimed, and for which a patent is sought on the invention entitled: **COMPUTATIONAL NUCLEIC ACID CODING AND FEATURE ANALYSIS**.

(C) The specification of which is attached.

(D) I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

(E) I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, § 1.56.

(F) I hereby appoint the following registered practitioners to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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Donna E. Scherer, Reg. No. 34,719
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Celine T. Callahan, Reg. No. 34,301
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J. Jay Guiliano, Reg. No. 41,810
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Robert C. Laurenson, Reg. No. 34,206
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Matthew J. Moore, Reg. No. 42,012
M. Todd Rands, Reg. No. 46,249
Richard San Pietro, Reg. No. 45,071
Charles Bret Seaton, Reg. No. 46,171
Michael J. Stimson, Reg. No. 45,429
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(H) **DECLARATION**: I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE

James D. McIninch
Inventor's signature James D. McIninch **Date** 10/26/2000
Country of Citizenship United States of America
Residence Burlington, MA, USA
Post Office Address 43 Beaverbrook Road
Burlington, MA 01803

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
James D. McININCH	Art Unit: To be assigned
Appl. No. To be assigned	Examiner: To be assigned
Filed: October 30, 2000	Atty. Docket: 04983.0220.00US00/ 38-10(15494)A
For: **Computational Nucleic Acid Coding and Feature Analysis**	

Statement Regarding Sequence Submission

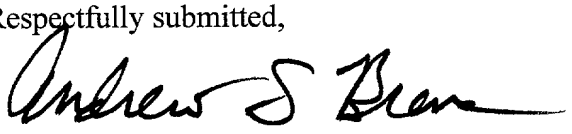
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above-mentioned application are the same.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

Respectfully submitted,



David R. Marsh (Reg. No. 41,408)
Andrew S. Brenc (Reg. No. 45,534)

Date: October 30, 2000

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SEQUENCE LISTING

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ctctcaatcc gaaagctaac cgtgagaaga tgactcagat catgtttgag acattcaata 360

ctcctgctat gtatgttgcc attcaagctg ttctctcact ctatgccagt ggccgtacta 420

ctggtcagta cattactaca ttctttttat accgtttggt tgaaataaaa ttcggtttgg 480

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ggagatggtg tgagccacac ggtaccaatc tacgagggtt atgcacttcc acacgcaatc 600

ctgcgtcttg atcttgcagg tcgtgaccta accgaccacc ttatgaaaat cctgacagag 660

cgtggttact ctttcaccac aactgctgag cgtgagattg ttagagacat gaaggagaag 720

ctctcttaca ttgccttgga ctttgaacaa gagctcgaga cttccaaaac aagctcatcc 780

gttgagaaga gcttcgagct gccagacggt caagtgatca ccatcggggc agagcgtttc 840

cgatgccctg aagttctgtt tcagccatcg atgatcggaa tggaaaatcc gggaattcat 900

gaaactactt acaactcaat catgaaatgt gatgtggata tcaggaagga tctttatgga 960

aacattgtgc ttagtggtgg caccacaatg ttcgatggga ttggtgatag gatgagtaaa 1020

gagatcacag cgttggctcc aagcagtatg aacatcaaag tggtggctcc accggaaagg 1080

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atttgtgttc ttttattctt gtccctcgtt gttcatttta atctcttttt tgcaacaaag 1440

caacttaaaa aaacagagca gtcattaaca gaatgttatt attatatata tgtatacata 1500

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caattttggg gatctcaaca agaacatgaa cttcaacttc tagtcatatg acgacctgag 1800

tctgcgcggc tgtgaatctc tttgctgcag taaatgttta caagtggtgt gtaaattggt 1860

actgattcaa aagctttaag aaatctacac atttcgtgaa attatttagc agacttgata 1920

ttaaaaatct aggataaaat gactatccaa agacaaatag gactgtttca catgttcccc 1980

tgattcttgt agctcataac tcatcagcag ttaacttttc tacctcatac acgctcgcaa 2040

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<211> 423
<212> PRT
<213> Unknown

<220>
<223> Describes a predicted protein sequence

<220>
<221> site
<222> (1)...(423)
<223> A stop codon is predicted at all XAA locations

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Xaa Arg Phe Phe Arg Ala Leu Xaa Ala Val Leu Ala Thr Pro Val Xaa
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Trp Leu Gly Trp Asp Lys Arg Met Leu Met Leu Glu Thr Arg Leu Asn
            20                  25                  30

Gln Asn Val Val Ser Xaa Leu Xaa Ser Thr Gln Leu Ser Met Glu Leu
        35                  40                  45

Leu Ile Ile Gly Met Thr Trp Arg Arg Phe Gly Ile Thr Leu Ser Thr
    50                  55                  60

Met Ser Phe Val Leu Pro Leu Lys Asn Ile Arg Xaa Leu Thr Glu Ala
65                  70                  75                  80

Pro Leu Asn Pro Lys Ala Asn Arg Glu Lys Met Thr Gln Ile Met Phe
                85                  90                  95

Glu Thr Phe Asn Thr Pro Ala Met Tyr Val Ala Ile Gln Ala Val Leu
            100                 105                 110

Ser Leu Tyr Ala Ser Gly Arg Thr Thr Gly Gln Tyr Ile Thr Thr Phe
        115                 120                 125

Phe Leu Tyr Arg Xaa Ser Gly Asp Gly Val Ser His Thr Val Pro Ile
    130                 135                 140

Tyr Glu Gly Tyr Ala Leu Pro His Ala Ile Leu Arg Leu Asp Leu Ala
145                 150                 155                 160

Gly Arg Asp Leu Thr Asp His Leu Met Lys Ile Leu Thr Glu Arg Gly
                165                 170                 175

Tyr Ser Phe Thr Thr Thr Ala Glu Arg Glu Ile Val Arg Asp Met Lys
            180                 185                 190

Glu Lys Leu Ser Tyr Ile Ala Leu Asp Phe Glu Gln Glu Leu Glu Thr
        195                 200                 205

Ser Lys Thr Ser Ser Ser Val Glu Lys Ser Phe Glu Leu Pro Asp Gly
    210                 215                 220

Gln Val Ile Thr Ile Gly Ala Glu Arg Phe Arg Cys Pro Glu Val Leu
225                 230                 235                 240

Phe Gln Pro Ser Met Ile Gly Met Glu Asn Pro Gly Ile His Glu Thr
                245                 250                 255
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Thr Tyr Asn Ser Ile Met Lys Cys Asp Val Asp Ile Arg Lys Asp Leu
260 265 270

Tyr Gly Asn Ile Val Leu Ser Gly Gly Thr Thr Met Phe Asp Gly Ile
275 280 285

Gly Asp Arg Met Ser Lys Glu Ile Thr Ala Leu Ala Pro Ser Ser Met
290 295 300

Lys Ile Lys Val Val Ala Pro Pro Glu Arg Lys Tyr Ser Val Trp Ile
305 310 315 320

Gly Gly Ser Ile Xaa Val Pro Asn Leu Gln Met Trp Ile Ala Lys Ala
325 330 335

Glu Tyr Xaa Asn Leu Asp Arg Gln Ser Ser Thr Gly Ser Ala Ser Asp
340 345 350

Gln Lys Ser Pro Ser Lys Thr Arg Ala Val Lys Ile Leu Xaa Asn Ser
355 360 365

Ser Ala Val Asn Phe Ser Thr Ser Tyr Thr Leu Ala Ile Arg Leu Glu
370 375 380

Leu Ser Ala Leu Ile Phe Leu Ile Ser Leu Glu Ile Ile Ser Ser Ser
385 390 395 400

Ile Lys Trp Gly Met Ala Ser Ser Ser Ile Cys Asn Ser Ser Lys Leu
405 410 415

Ser Met Lys Lys Gln Ser Xaa
420

<210> 3
<211> 422
<212> PRT
<213> Unknown

<220>
<223> Describes a predicted protein sequence

<220>
<221> site
<222> (1)...(422)
<223> A stop codon is predicted at all XAA locations

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Xaa Arg Phe Phe Arg Ala Leu Xaa Ala Val Leu Ala Thr Pro Val Xaa
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Trp Leu Gly Trp Asp Lys Arg Met Leu Met Leu Glu Thr Arg Leu Asn

20 25 30

Gln Asn Val Val Ser Xaa Leu Xaa Ser Thr Gln Leu Ser Met Glu Leu
35 40 45

Leu Ile Ile Gly Met Thr Trp Arg Arg Phe Gly Ile Thr Leu Ser Thr
50 55 60

Met Ser Phe Val Leu Pro Leu Lys Asn Ile Arg Xaa Leu Thr Glu Ala
65 70 75 80

Pro Leu Asn Pro Lys Ala Asn Arg Glu Lys Met Thr Gln Ile Met Phe
85 90 95

Glu Thr Phe Asn Thr Pro Ala Met Tyr Val Ala Ile Gln Ala Val Leu
100 105 110

Ser Leu Tyr Ala Ser Gly Arg Thr Thr Gly Gln Tyr Ile Thr Thr Phe
115 120 125

Phe Leu Tyr Arg Xaa Ser Gly Asp Gly Val Ser His Thr Val Pro Ile
130 135 140

Tyr Glu Gly Tyr Ala Leu Pro His Ala Ile Leu Arg Leu Asp Leu Ala
145 150 155 160

Gly Arg Asp Leu Thr Asp His Leu Met Lys Ile Leu Thr Glu Arg Gly
165 170 175

Tyr Ser Phe Thr Thr Thr Ala Glu Arg Glu Ile Val Arg Asp Met Lys
180 185 190

Glu Lys Leu Ser Tyr Ile Ala Leu Asp Phe Glu Gln Glu Leu Glu Thr
195 200 205

Ser Lys Thr Ser Ser Ser Val Glu Lys Ser Phe Glu Leu Pro Asp Gly
210 215 220

Gln Val Ile Thr Ile Gly Ala Glu Arg Phe Arg Cys Pro Glu Val Leu
225 230 235 240

Phe Gln Pro Ser Met Ile Gly Met Glu Asn Pro Gly Ile His Glu Thr
245 250 255

Thr Tyr Asn Ser Ile Met Lys Cys Asp Val Asp Ile Arg Lys Asp Leu
260 265 270

Tyr Gly Asn Ile Val Leu Ser Gly Gly Thr Thr Met Phe Asp Gly Ile
275 280 285

Gly Asp Arg Met Ser Lys Glu Ile Thr Ala Leu Ala Pro Ser Ser Met
290 295 300

Lys Ile Lys Val Val Ala Pro Pro Glu Arg Lys Tyr Ser Val Trp Ile

305 310 315 320

Gly Gly Ser Ile Leu Ala Ser Xaa Gln Met Trp Ile Ala Lys Ala Glu
325 330 335

Tyr Xaa Asn Leu Asp Arg Gln Ser Ser Thr Gly Ser Ala Ser Asp Gln
340 345 350

Lys Ser Pro Ser Lys Thr Arg Ala Val Lys Ile Leu Xaa Asn Ser Ser
355 360 365

Ala Val Asn Phe Ser Thr Ser Tyr Thr Leu Ala Ile Arg Leu Glu Leu
370 375 380

Ser Ala Leu Ile Phe Leu Ile Ser Leu Glu Ile Ile Ser Ser Ser Ile
385 390 395 400

Lys Trp Gly Met Ala Ser Ser Ser Ile Cys Asn Ser Ser Lys Leu Ser
405 410 415

Met Lys Lys Gln Ser Xaa
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<211> 296
<212> PRT
<213> Arabidopsis thaliana

<220>
<223> Ecotype columbia, describes actin

<400> 4

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Pro Glu Glu His Pro Val Leu Leu Thr Glu Ala Pro Leu Asn Pro Lys
20 25 30

Ala Asn Arg Glu Lys Met Thr Gln Ile Met Phe Glu Thr Phe Asn Thr
35 40 45

Pro Ala Met Tyr Val Ala Ile Gln Ala Val Leu Ser Leu Ala Ser Gly
50 55 60

Arg Thr Thr Gly Gly Ile Val Leu Asp Ser Gly Asp Gly Val Ser His
65 70 75 80

Thr Val Pro Ile Tyr Glu Gly Tyr Ala Leu Pro His Ala Ile Leu Arg
85 90 95

Leu Asp Leu Ala Gly Arg Asp Leu Thr Asp His Leu Met Lys Ile Leu
100 105 110

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Thr Glu Arg Gly Tyr Ser Phe Thr Thr Thr Ala Glu Arg Glu Ile Val
        115                 120                 125

Arg Asp Met Lys Glu Lys Leu Ser Tyr Ile Ala Leu Asp Phe Glu Gln
    130                 135                 140

Glu Leu Glu Thr Ser Lys Thr Ser Ser Ser Val Glu Lys Ser Phe Glu
145                 150                 155                 160

Leu Pro Asp Gly Gln Val Ile Thr Ile Gly Ala Glu Arg Phe Arg Cys
                165                 170                 175

Pro Glu Val Leu Phe Gln Pro Ser Met Ile Gly Met Glu Asn Pro Gly
            180                 185                 190

Ile His Glu Thr Thr Tyr Asn Ser Ile Met Lys Cys Asp Val Asp Ile
        195                 200                 205

Arg Lys Asp Leu Tyr Gly Asn Ile Val Leu Ser Gly Gly Thr Thr Met
    210                 215                 220

Phe Gly Gly Ile Gly Asp Arg Met Ser Lys Glu Ile Thr Ala Leu Ala
225                 230                 235                 240

Pro Ser Ser Met Lys Ile Lys Val Val Ala Pro Pro Glu Arg Lys Tyr
                245                 250                 255

Ser Val Trp Ile Gly Gly Ser Ile Leu Ala Ser Leu Ser Thr Phe Gln
            260                 265                 270

Gln Met Gln Met Trp Ile Ala Lys Ala Glu Tyr Asp Glu Ser Gly Pro
        275                 280                 285

Ser Ile Val His Arg Lys Cys Phe
    290                 295
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